National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

UNIVERSITY OF ALBERTA

EFFECT OF A COLD ENVIRONMENT ON PROTEIN SYNTHESIS AND ENDOCRINE KINETICS IN GROWING CATTLE

BY

SHANNON LOUISE SCOTT ©

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

ANIMAL PHYSIOLOGY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1991

National Library of Canada
Canadian Theses Service
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Service des thèses canadiennes

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-66776-1

Canada

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Shannon Louise Scott

TITLE OF THESIS: Effect of a Cold Environment on Protein Synthesis and Endocrine Kinetics in Growing Cattle.

DEGREE: Doctor of Philosophy

YEAR THIS DEGREE GRANTED: 1991

Permission is hereby granted to the UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(signed) Shannon L Scott

PERMANENT ADDRESS:

Site 4, Box 17, RR#1
South Edmonton
Alberta, T6H 4N6

Date: April 22, 1991

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Effect of a Cold Environment on Protein Synthesis and Endocrine Kinetics in Growing Cattle submitted by Shannon Louise Scott in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Physiology.

R.J. CHRISTOPHERSON (supervisor)

JR Thompson

J.R. THOMPSON

V.E. BARACOS

C.R. KRISHNAMURTI

Michael I. McBurney

M.I. McBURNEY

Date: April 22, 1991

ABSTRACT

Two studies were conducted to assess the effects of cold-adaptation on protein synthesis and endocrine kinetics in growing cattle. In the first study, eleven Holstein bull calves 35 days of age were assigned to one of three treatment groups: 1) W72: warm environment (20°C), 72 g feed $kg^{-0.75}BW$ day^{-1}, 2) C72: cold environment (-5°C), 72 g feed $kg^{-0.75}BW$ day^{-1}, or 3) C90: cold environment (-5°C), 90 g feed $kg^{-0.75}BW$ day^{-1}. Fractional synthesis rates of protein in the rumen, rumen papillae, omasum, intestine (duodenum), kidney, liver, heart, longissimus dorsi, biceps femoris, and perineal skin were determined following a continuous infusion of $[^3H]$-phenylalanine. Rates in the two muscles and skin were reduced along with nitrogen retention in the calves in the C72 group. Muscle protein degradation, estimated from urinary N^{τ}-methylhistidine excretion, tended to be elevated in the C72 and C90 groups, resulting in reduced muscle protein gain in the C72 group. Increased protein turnover did not appear to contribute to the increase in heat production in the cold.

In a second experiment, ten Holstein heifers one year of age were housed in groups of five in temperature-controlled environmental chambers. They were assigned to either a warm (20 °C) or cold (-17 °C) environment. Each heifer received an injection of insulin or growth hormone followed by frequent blood sampling. Plasma samples were analyzed for insulin, insulin-like growth factor I (IGF-I), growth hormone, glucagon, triiodothyronine, thyroxine and glucose. Basal plasma concentrations of all hormones except growth hormone and IGF-I were elevated in the cold environment. Both the zero-time intercept and the rate constant for the slow pool of growth hormone were decreased in cold-adapted animals. Plasma glucose concentration was higher in the cold-adapted heifers during the response to both hormone injections. Glucagon and IGF-I responses to insulin and growth hormone injection, respectively, were greater in the cold.

These results demonstrate that both feed intake and hormones may regulate changes in protein metabolism in cold-adapted animals.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor, Dr. R.J. Christopherson, for his guidance and support throughout my Doctoral program. I would also like to thank Dr. V.E. Baracos and Dr. J.R. Thompson for their insight and encouragement as members of my graduate committee.

I would like to acknowledge the diligent and cheerful technical assistance of F. Hoeksema, L. Makowecki, N. Robinson, G. Sedgwick, and the staff of the Metabolic Unit, J. Francis, C. Gorsak, and P. Gregory. I am also grateful to the many staff and students whose generous gifts of time and friendship have made my way a little easier. I would also like to recognize the excellent advice of R. Weingardt regarding statistical analyses.

I would like to thank my husband Dave for his patience and encouragement throughout my program. I am indebted to my parents for their contributions. To my mother, who taught me perseverance, and to my father, who first introduced me to the intriguing world of science.

Financial support from the Natural Sciences and Engineering Research Council of Canada and the Province of Alberta are greatly appreciated.

TABLE OF CONTENTS

CHAPTER PAGE

I. INTRODUCTION.. 1

LITERATURE CITED.................................. 9

II. EFFECT OF A COLD ENVIRONMENT ON PROTEIN SYNTHESIS AND METABOLISM IN CALVES.................................. 12

LITERATURE CITED.................................. 58

III. EFFECT OF A COLD ENVIRONMENT ON ENDOCRINE PARAMETERS IN HEIFERS AND CALVES.................................. 62

LITERATURE CITED.................................. 107

IV. GENERAL DISCUSSION AND CONCLUSIONS.................... 110

LITERATURE CITED.................................. 132

APPENDIX 1.. 137

APPENDIX 2.. 138

APPENDIX 3.. 139

APPENDIX 4.. 140

APPENDIX 5.. 141

APPENDIX 6.. 142

APPENDIX 7.. 143

APPENDIX 8.. 144

APPENDIX 9.. 150

APPENDIX 10.. 165

APPENDIX 11.. 174

LIST OF TABLES

TABLE PAGE

TABLE II-1 Composition of diet 38

TABLE II-2 Average body weight, average daily intake, average daily gain, gain/feed (% efficiency), heat production and crude protein retention of calves adapted to two environmental temperatures 39

TABLE II-3 Absolute synthesis rate of protein (g protein day^{-1}) in tissues from calves adapted to two environmental temperatures 40

TABLE II-4 Protein synthetic activity of RNA (mg protein synthesized day^{-1} mg RNA^{-1}) in tissues from calves adapted to two environmental temperatures 41

TABLE II-5 Average phenylalanine flux, whole-body protein synthesis, whole-body protein gain, and whole-body protein degradation kg^{-1} body weight of calves adapted to two environmental temperatures 42

TABLE II-6 Muscle protein synthesis, muscle protein degradation, and muscle protein gain kg^{-1} body weight of calves adapted to two environmental temperatures 43

LIST OF TABLES (continued)

TABLE PAGE

TABLE III-1 Basal (pre-injection) concentrations of insulin, glucagon, growth hormone, triiodothyronine (T_3), thyroxine (T_4), and glucose in heifers adapted to warm and cold environments.................................. 86

TABLE III-2 Insulin kinetic parameters in heifers adapted to warm and cold environments................. 87

TABLE III-3 Growth hormone kinetic parameters in heifers adapted to warm and cold environments.. 88

TABLE III-4 Growth hormone parameters of calves adapted to two environmental temperatures.................... 89

TABLE III-5 Basal triiodothyronine (T_3), thyroxine (T_4), and insulin-like growth factor I of calves adapted to two environmental temperatures............. 90

TABLE IV-1 A summary of the fractional synthesis rate (FSR; % day^{-1}) of protein in skin and the contribution of the absolute rate of protein synthesis in skin to whole-body synthesis (%) in several animal species.............. 129

LIST OF FIGURES

FIGURE PAGE

FIGURE II-1 The rise in plasma phenylalanine specific radioactivity during an 8 hour infusion of ^{3}H-phenylalanine.................................. 45

FIGURE II-2A The fractional synthesis rate (% day^{-1}) of protein in muscle from calves adapted to two environmental temperatures...................... 47

FIGURE II-2B The fractional synthesis rate (% day^{-1}) of protein in kidney and skin from calves adapted to two environmental temperatures........... 49

FIGURE II-2C The fractional synthesis rate (% day^{-1}) of protein in liver and heart from calves adapted to two environmental temperatures........... 51

FIGURE II-2D The fractional synthesis rate (% day^{-1}) of protein in rumen wall and rumen papillae from calves adapted to two environmental temperatures...................................... 53

FIGURE II-2E The fractional synthesis rate (% day^{-1}) of protein in intestine and omasum from calves adapted to two environmental temperatures........... 55

FIGURE II-3 The relationship between the daily intake of nitrogen (g N day^{-1}) and absolute rates of protein synthesis in muscle or skin (g N day^{-1}).................................... 57

LIST OF FIGURES (continued)

FIGURE PAGE

FIGURE III-1 The insulin disappearance curve for heifer 603 in the cold environment in Period 2 after a single intravenous injection.......... 92

FIGURE III-2 Effect of insulin injection on plasma glucose concentration (mg mL^{-1}) in heifers adapted to warm and cold environments.......... 94

FIGURE III-3 Effect of insulin injection on plasma glucagon concentration (pg mL^{-1}) in heifers adapted to warm and cold environments.......... 96

FIGURE III-4 Effect of insulin injection on plasma growth hormone concentration (ng mL^{-1}) in heifers adapted to warm and cold environments.......... 98

FIGURE III-5 Effect of growth hormone injection on plasma glucose concentration (mg mL^{-1}) in heifers adapted to warm and cold environments.......... 100

FIGURE III-6 Effect of growth hormone injection on plasma insulin concentration (ng mL^{-1}) in heifers adapted to warm and cold environments.......... 102

LIST OF FIGURES (continued)

FIGURE PAGE

FIGURE III-7 Effect of growth hormone injections at time 0 hours and time 24 hours on serum insulin-like growth factor I concentration (ng mL^{-1}) in heifers adapted to warm and cold environments.................................... 104

FIGURE III-8 An example of pulsatile growth hormone release during an 8 hour continuous infusion of ^{3}H-phenylalanine in calf 832 in the W72 group.................................... 106

FIGURE IV-1 The relationship between RNA content (mg RNA g^{-1} protein) and the fractional synthesis rate of protein (% day^{-1}) in calves adapted to two environments.................. 131

CHAPTER I

GENERAL INTRODUCTION

Effect of a Cold Environment on Metabolism

A variety of metabolic responses to cold-adaptation result in increased use of metabolizable energy for thermoregulatory heat production to prevent a fall in core body temperature. Cold-adaptation can thus alter the efficiency of production, possibly resulting in economic losses. Information regarding the nature of these metabolic alterations could also indicate potential sources of production losses. Past research has shown that metabolic responses to cold-adaptation include changes in food intake, growth, the endocrine system, and, perhaps, protein turnover.

During cold-adaptation, food intake increases (Webster, Hicks, and Hays, 1969). There is also an increased rate of passage of digesta through the digestive tract independent of changes in food intake (Westra and Christopherson, 1976). Therefore, in investigations on the effects of cold-adaptation on metabolic parameters, it is necessary to distinguish between effects actually due to the cold environment and those due to feed intake. For example, increased thermogenesis in the cold could be due to increased food intake or to an effect of cold-adaptation itself. Young (1975) demonstrated that even when feed intake was held constant, thermoneutral metabolic rate was higher in cattle adapted to -10°C than 20°C.

Cold-adaptation usually requires increased use of substrates for thermogenesis, whether or not food intake increases (Sasaki and Weekes, 1985). For example, in shorn sheep adapted to 0°C for 7-23 days and fed the same as when they were at 20°C, glucose flux was increased (Weekes *et al.*, 1983). In shorn ewes fed 20% more at -2°C than at 18°C during a six week adaptation period, the glucose flux and percent of glucose oxidized to CO_2 was also increased (M^{c}Kay *et al.*, 1974). Amino acids may comprise a portion of glucose flux by contributing carbon skeletons to gluconeogenesis (Lindsay, 1976), although the extent of this process in the cold is unknown.

The endocrine system likely plays a role in coordinating metabolic responses to a cold environment. Because insulin is an important anabolic hormone, effects of cold on insulin secretion as well as tissue sensitivity and responsiveness have been studied extensively. After finding that cold exposure (0°C for 4-19 days) reduced insulin secretory response to glucose injection in sheep (Sasaki *et al*., 1982), Weekes *et al.* (1983) conducted further studies showing that the responsiveness of glucose utilization to insulin was enhanced in sheep exposed to 0°C for 4-23 days compared with 20°C.

Growth hormone concentration may also be altered in the cold. In three cows adapted to -26°C for 3 weeks, plasma growth hormone was increased in one animal during cold exposure, and in all animals for three weeks following a return to thermoneutral temperatures (Olsen and Trenkle, 1973). Effects of growth hormone are mediated by the somatomedins, such as insulin-like growth factor I (IGF-I; Zapf and Froesch, 1986). Plasma IGF-I concentrations were depressed in young pigs acclimated to 10°C compared with 35°C, especially if they were on a low level of intake Dauncey *et al.*, 1990).

Some catabolic hormones that may be involved in adaptation to a cold environment include catecholamines, thyroid hormones, and glucagon. In sheep maintained at 2-5 °C for at least 28 days, plasma catecholamines (adrenaline and noradrenaline) were elevated compared with animals kept at 22-25°C (Christopherson *et al.*, 1978). In the same experiment, thyroid hormones (thyroxine and triiodothyronine) were also elevated by chronic cold exposure. There appeared to be a synergism between catecholamines and thyroid hormones since thyroidectomized sheep receiving intramuscular injections of triiodothyronine showed a smaller response in plasma adrenaline to acute cold exposure than untreated sheep. Catecholamines also appear to decrease insulin secretion and increase glucagon secretion in sheep in the cold via an α-adrenergic effect on the islets of Langerhans in the pancreas (Sasaki *et al.*, 1982).

Effects of Cold on Protein Metabolism and Protein Turnover

Although it is artificial to discuss protein metabolism separately from energy metabolism, especially in ruminants (MacRae and Reeds, 1980), effects of cold on nitrogen metabolism have been reported. Wethers fed a pelleted grass diet exhibited reduced nitrogen retention at 0.8°C compared with 18° C (Westra and Christopherson, 1976). In lambs, nitrogen retention was quadratically related to temperature, dropping down at lower temperatures (Ames and Brink, 1977). Ames *et al.* (1980) suggested that in a cold environment, dietary protein is being used as an energy source to meet increased maintenance energy, therefore reducing protein available for tissue synthesis and growth. The same researchers also showed that if a portion of dietary protein was replaced with an energy source, the remaining protein could be used more efficiently for growth.

In order for protein deposition to occur, protein synthesis must exceed protein degradation. Together, the latter two processes comprise protein turnover. Little information is available on the effect of environmental temperature on the rate of protein turnover, particularly for domestic species. Aoyagi *et al.* (1988) found that in chicks exposed to a low ambient temperature (22°C), whole-body protein synthesis and degradation were increased compared to 30°C. Lindsay *et al.* (1988) showed that tyrosine catabolic rate was elevated in pigs acclimated to 10°C compared with 35°C, although environment did not influence rates of whole-body or muscle protein synthesis. Work by Thompson *et al.* (1987) indicated that in restricted-fed calves acutely exposed to a temperature of -10°C, urinary excretion of N^{τ}-methylhistidine was 35% higher than in calves maintained in a thermoneutral environment, suggesting increased myofibrillar protein breakdown. Since more than 90% of the whole-body N^{τ}-methylhistidine is found in muscle (Nishizawa *et al.*, 1979), it was assumed that this represented an increase in skeletal muscle protein degradation in the cold environment. No estimation of protein synthesis was made in that study. Nevertheless, some experimental results suggest that protein synthesis and/or degradation may be altered by adaptation to a cold environment.

Hormonal Control of Protein Turnover

Although many hormones have been examined as regulators of protein turnover, attention has been focused on growth hormone, insulin, and ß-adrenergic agonists. Both collagen and non-collagen protein exhibit increased fractional synthesis rates in muscles from lambs receiving daily injections of growth hormone, most likely due to observed increases in RNA content (Pell and Bates, 1987). However, the biceps femoris muscle, predominantly a red fiber type, appeared to be more sensitive to growth hormone treatment than did the mainly white fiber type semitendinosus muscle, which did not exhibit significant increases in either collagen or non-collagen protein synthesis. These findings were supported by findings of Eisemann *et al.* (1989) who found an elevated fractional synthesis rate of insoluble protein in several muscles except the semitendinosus in growing steers treated with somatotropin for 20 days. In addition, whole body protein synthesis was increased and leucine oxidation decreased. However, in steers treated with recombinant growth hormone for a longer length of time (112 days), there were no significant effects of treatment on fractional synthesis rates of protein in any tissue (M^{c}Bride *et al.*, 1989). These results may indicate a decreased responsiveness to growth hormone treatment with time.

Considering the central role of insulin in amino acid transport and cellular anabolism, it has also been postulated to influence protein synthesis. In sheep, an infusion of insulin plus glucose had no effect on the fractional synthesis rate of protein in liver, kidney, gastrocnemius muscle, or external intercostal muscle (Early *et al.*, 1988). Lack of a response to insulin may have been due to the fed state of the animals, as insulin infusion increased muscle protein synthesis in postabsorptive but not fed rats (Garlick *et al.*, 1983).

Repartitioning agents, such as the ß-adrenergic agonists cimaterol and clenbuterol, are named for their ability to increase protein accretion while reducing fat accretion (Kim *et al.*, 1989). Feeding cimaterol to lambs also improved nitrogen retention, mainly by decreasing urinary nitrogen, without a concomitant increase in nitrogen intake (Kim *et al.*, 1989). Treatment with another ß-agonist, clenbuterol, had a similar effect on nitrogen retention in lambs on fixed feed intake (MacRae *et al.*, 1988). In that experiment, although leucine oxidation was reduced, whole body protein synthesis did not change, indicating a reduction in protein degradation. Clenbuterol also elevated nitrogen retention in the carcass of veal calves without affecting non-carcass components (Williams *et al.*, 1987). Effects of clenbuterol were most evident in muscle, including a reduction in the fractional breakdown rate of muscle protein estimated from urinary excretion of N^{τ}-methylhistidine.

Although clenbuterol treatment increased the weight and fractional rate of gain of muscle protein in the longissimus dorsi muscle of wether lambs, there was no corresponding effect on the fractional synthesis rate of protein, again suggesting a reduction in protein degradation (Bohorov *et al.*, 1987). In addition, there was no effect of clenbuterol treatment on tyrosine flux, indicating there was no change in whole-body protein synthesis. However, no data for the proportion of tyrosine flux going to oxidation were given in that experiment, so no definite statement can be made about the effect of clebuterol on protein synthesis. A 9-day treatment of growing steers with clenbuterol resulted in increased net uptake of α-NH_2 nitrogen by the hindquarters, consistent with increased skeletal protein accretion (Eisemann *et al.*, 1988). In summary, it appears that ß-adrenergic agonists increase protein accretion mainly by depressing protein degradation rather than by elevating protein synthesis.

Other hormones such as thyroid hormones and glucocorticoids play a role in regulating protein turnover in rats (Millward *et al.*, 1983), but they have not been examined in any great detail in ruminants. In a preliminary study by Crompton and Lomax (1987), the relationship of plasma concentrations of growth hormone, insulin, thyroxine, and cortisol to protein synthesis in the hind-limb of lambs following undernourishment and refeeding was examined. Increased protein synthesis following refeeding was associated with a rise in insulin concentration and a fall in growth hormone and cortisol levels. While protein synthesis was positively related to insulin and thyroxine concentrations and negatively related to growth hormone concentration, protein degradation was positively related to cortisol concentration. In fed sheep injected daily with thyroxine for five weeks, a similar relationship was observed between plasma free triiodothyronine concentration and *in vitro* rates of muscle and hepatocyte protein synthesis (M^{c}Bride and Early, 1989).

Taking into account the established effects of cold-adaptation on thermogenesis and the positive relationship observed between heat production and protein synthesis (Webster, 1983), it is possible that increased protein synthesis may contribute to cold-induced thermogenesis. Since *ad libitum* intake usually increases during cold-adaptation, feed intake may affect the response of protein synthesis and nitrogen metabolism to cold-adaptation. Since cold-adaptation can influence plasma concentrations of both protein anabolic and catabolic hormones, it was hypothesized that it may also alter hormonal kinetic parameters. Since several hormones have been demonstrated to regulate protein synthesis, endocrine alterations may stimulate protein synthesis in the cold to increase heat production.

With these hypotheses in mind, the research in this thesis was designed to evaluate the effect of cold-adaptation and feed intake on protein metabolism and fractional synthesis rates of protein in several tissues from calves. A further objective was to determine the contribution, if any, of protein synthesis to cold-induced thermogenesis. Experiments were also designed to ascertain the response of plasma concentrations of anabolic and catabolic hormones in heifers to cold-adaptation. A further objective was to evaluate the effect of cold-adaptation on insulin and growth hormone kinetic parameters observed following a single hormone injection and on the responses of other hormones and glucose to those injections. A final objective was to correlate endocrine responses to cold-adaptation in heifers with changes in protein synthesis in calves to elucidate regulatory mechanisms. Results from these studies will provide information on metabolic responses to cold-adaptation that may affect animal production in cold environments.

LITERATURE CITED

Ames, D.R., and D.R. Brink. 1977. Effect of temperature on lamb performance and protein efficiency ratio. J. Anim. Sci. 44: 136-140.

Ames, D.R., Brink, D.R., and C.L. Willms. 1980. Adjusting protein in feedlot diets during thermal stress. J. Anim. Sci. 50: 1-6.

Aoyagi, Y., Tasaki, I., Okumura, J.-I., and T. Muramatsu. 1988. Effect of low ambient temperature on protein turnover and heat production in chicks. Comp. Biochem. Physiol. 89A: 433-436.

Bohorov, O., Buttery, P.J., Correia, J.H.R.D., and J.B. Soar. 1987. The effect of the ß-2-adrenergic agonist clenbuterol or implantation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. Br. J. Nutr. 57: 99-107.

Christopherson, R.J., Thompson, J.R., Hammond, V.A., and G.A. Hills. 1978. Effects of thyroid status on plasma adrenaline and noradrenaline concentrations in sheep during acute and chronic cold exposure. Can. J. Physiol. Pharmacol. 56: 490-496.

Crompton, L.A., and M.A. Lomax. 1987. The relation between hind-limb muscle protein metabolism and growth hormone, insulin, thyroxine, and cortisol in growing lambs. Proc. Nutr. Soc. 46: 45A.

Dauncey, M.J., Shakespear, R.A., Rudd, B.T., and D.L. Ingram. 1990. Variations in somatomedin-C/insulin-like growth factor-I associated with environmental temperature and nutrition. Horm. Metab. Res. 22: 261-264.

Early, R.J., M^{c}Bride, B.W., and R.O. Ball. 1988. Phenylalanine metabolism in sheep infused with glucose plus insulin. II: Effects on in vivo and in vitro protein synthesis and related energy expenditures. Can. J. Anim. Sci. 68: 721-730.

Eisemann, J.H., Hammond, A.C., and T.S. Rumsey. 1989. Tissue protein synthesis and nucleic acid concentrations in steers treated with somatotropin. Br. J. Nutr. 62: 657-671.

Eisemann, J.H., Huntington, G.B., and C.L. Ferrell. 1988. Effects of dietary clenbuterol on metabolism of the hindquarters in sheep. J. Anim. Sci. 66: 342-353.

Garlick, P.J., Fern, M., and V.R. Preedy. 1983. The effect of insulin infusion and food intake on muscle protein synthesis in postabsorptive rats. Biochem. J. 210: 669-676.

Kim, Y.S., Lee, Y.B., Garrett, W.N., and R.H. Dalrymple. 1989. Effects of cimaterol on nitrogen retention and energy utilization in lambs. J. Anim. Sci. 67: 674-681.

Lindsay, D.B. 1976. Amino acids as sources of energy. In: Protein Metabolism and Nutrition, D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale, and H. Swan, eds., pp. 183-184. EAAP Publication No. 16. Butterworths, London.

Lindsay, D.B., Dauncey, M.J., Barker, P.J., and D.L. Ingram. 1988. Protein synthesis and degradation after acclimation to different environmental temperatures and energy intakes. J. Therm. Biol. 13: 79-83.

MacRae, J.C., and P.J. Reeds. 1980. Prediction of protein deposition in ruminants. In: Protein Deposition in Animals, P.J. Buttery and D.B. Lindsay, eds., pp. 225-249. Butterworths, London.

MacRae, J.C., Skene, P.A., Connell, A., Buchan, V., and G.E. Lobley. 1988. The action of the ß-agonist clenbuterol on protein and energy metabolism in fattening wether lambs. Br. J. Nutr. 59: 457-465.

M^{c}Bride, B.W., Early, R.J., and R.O. Ball. 1989. Protein synthesis and the energy costs of Na^{+}, K^{+}-transport in tissues of somatotropin treated steers. In: Energy Metabolism of Farm Animals, Y. van der Honing and W.H. Close, eds., pp.107-110. EAAP publication No. 43. Pudoc, Wageningen, Holland.

M^{c}Bride, B.W., and R.J. Early. 1989. Energy expenditure associated with sodium/potassium transport and protein synthesis in skeletal muscle and isolated hepatocytes from hyperthyroid sheep. Br. J. Nutr. 62: 673-682.

M^{c}Kay, D.G., Young, B.A., and L.P. Milligan. 1974. Energy substrates for cold induced thermogenesis. In Energy Metabolism of Farm Animals, K.H. Menke, H.-J. Lantzsch, and J.R. Reichl, eds. EAAP Publication No. 14. Universität Hohenheim Dokumentationsstelle, Stuttgart, Germany.

Millward, D.J., Bates, P.C., deBenoist, B., Brown, J.G., Cox, M., Halliday, D., Odedra, B., and M.J. Rennie. 1983. Protein turnover: The nature of the phenomenon and its physiological regulation. In: IVth International Symposium on Protein Metabolism and Nutrition, M. Arnal, R. Pion, and D. Bonin, eds.,pp. 69-96. EAAP Publication No. 31. INRA, Paris, France.

Nishizawa, N., Toyoda, Y., Noguchi, T., Hareyama, S., Itabashi, H., and R. Funabiki. 1979. N$^{\tau}$-Methylhistidine content of organs and tissues of cattle and an attempt to estimate fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth by measuring urinary output of N$^{\tau}$-methylhisitidine. Br.J. Nutr. 42: 247-252.

Olsen, J.D., and A. Trenkle. 1973. Exposure of cattle to controlled subzero temperature: growth hormone, glucose, and free fatty acid concentrations in plasma. Am. J. Vet. Res. 34: 747-751.

Pell, J.M., and P.C. Bates. 1987. Collagen and non-collagen protein turnover in skeletal muscle of growth hormone-treated lambs. J. Endocr. 115: R1-R4.

Sasaki, Y., Takahashi, H., Aso, H., Ohneda, A., and T.E.C. Weekes. 1982. Effects of cold exposure on insulin and glucagon secretion in sheep. Endocrinology 111: 2070-2076.

Sasaki, Y., and T.E.C. Weekes. 1985. Metabolic responses to cold. In: Control of Digestion and Metabolism in Ruminants, L.P. Milligan, W.L. Grovum, and A. Dobson, eds. Chapter 17. Reston Publishing Co., Reston, USA.

Thompson, J.R., Christopherson, R.J., and R.J. Early. 1987. Cold environmental temperatures increase the rate of skeletal muscle protein breakdown. 66th Annual Feeder's Day Report, p. 52-54. Agriculture and Forestry Bulletin, University of Alberta, Edmonton, Canada.

Webster, A.J.F. 1983. Energetics of maintenance and growth. In: Mammalian Thermogenesis, L. Girardier and M.J. Stock, eds., pp. 178-207. Chapman Hall Ltd., London.

Webster, A.J.F., Hicks, A.M., and F.L. Hays. 1969. Cold climate and cold temperature induced changes in heat production and thermal insulation of sheep. Can. J. Physiol. Pharmacol. 47: 553-562.

Weekes, T.E.C., Sasaki, Y., and T. Tsuda. 1983. Enhanced responsiveness to insulin in sheep exposed to cold. Am. J. Physiol. 244: E335-E345.

Westra, R., and R.J. Christopherson. 1976. Effects of cold on digestibility, retention time of digesta, reticulum motility and thyroid hormones in sheep. Can. J. Anim. Sci. 56: 699-708.

Williams, P.E.V., Pagliani, L., Innes, G.M., Pennie, K., Harris, C.I., and P. Garthwaite. 1987. Effects of a ß-agonist (clenbuterol) on growth, carcass composition, protein and energy metabolism of veal calves. Br. J. Nutr. 57: 417-428.

Young, B.A. 1975. Temperature-induced changes in metabolism and body weight of cattle (*Bos taurus*). Can J. Physiol. Pharmacol. 53: 947-953.

Zapf, J., and E.R. Froesch. 1986. Insulin-like factors/somatomedins: Structure, secretion, biological actions and physiological role. Horm. Res. 24: 121-130.

CHAPTER II

THE EFFECT OF A COLD ENVIRONMENT ON PROTEIN SYNTHESIS AND PROTEIN METABOLISM IN CALVES

INTRODUCTION

In regions where temperatures fall steeply in the winter months, significant energy losses occur due to cold-induced thermogenesis as animals increase heat production to maintain body temperature. It has been suggested that protein turnover is a thermogenic substrate cycle, accounting for about 20% of heat production (Newsholme, 1987). Therefore, increased protein turnover may be a part of cold-induced thermogenesis, affecting rates of protein synthesis and/or protein degradation in one or more tissues. Protein deposition occurs when protein synthesis exceeds degradation. Therefore, a change in the rate of either one as a result of adaptation to a cold environment may have an effect on production.

Little information is available on the effect of environmental temperature on the rate of protein turnover, particularly for domestic species. Aoyagi *et al.* (1988) found that in chicks exposed to a low ambient temperature (22°C), whole-body protein synthesis and degradation were increased compared to 30°C. In experiments with young pigs, Lindsay *et al.* (1988) showed that tyrosine catabolic rate was elevated in pigs acclimated to 10°C compared with 35°C, although environmental temperature did not influence rates of whole-body or muscle protein synthesis. Work by Thompson *et al.* (1987) indicated that urinary excretion of N^{τ}-methylhistidine was 35% higher in restricted-fed calves acutely exposed to a temperature of -10°C than in calves maintained in a thermoneutral environment, indicating increased myofibrillar protein breakdown. Since more than 90% of the whole-body N^{τ}-methylhistidine is found in muscle (Nishizawa *et al.*, 1979), it was assumed that this represented an increase in skeletal muscle protein degradation in the cold environment. Protein synthesis was not measured in this study. Therefore, there are some experimental results which suggest that protein synthesis and or degradation may be altered by adaptation to a cold environment.

Chronic exposure of animals to a cold environment usually leads to increased *ad libitum* feed intake. Even with an acute cold exposure, there was a greater change in urinary excretion of N^{τ}-methylhistidine when calves were fed *ad libitum* than when restricted-fed (Thompson *et al.*, 1987). Possibly, level of feed intake may also play a role in the effect of adaptation to a cold environment on protein turnover.

Since protein synthesis is a thermogenic process, it was hypothesized by Yousef and Chaffee (1970) that increased rates of tissue protein synthesis could contribute to heat production in cold-adapted calves; feed intake may affect this response. The objectives of the present study were to examine the effect of adaptation to a cold environment on the fractional synthesis rate of protein in several different tissues in growing calves, as well as to estimate muscle protein degradation.

MATERIALS AND METHODS

ANIMALS AND MANAGEMENT:

Eleven Holstein bull calves 35 days of age housed in individual metabolism crates in temperature-controlled chambers were randomly assigned to one of three treatment groups: 1) W72: warm environment (20°C), 72 g feed $kg^{-0.75}BW\ day^{-1}$, 2) C72: cold environment (-5°C), 72 g feed $kg^{-0.75}BW\ day^{-1}$, or 3) C90: cold environment (-5°C), 90 g feed $kg^{-0.75}BW\ day^{-1}$. Initially, there were twelve calves, but one had a severe navel infection and was removed from the experiment. The lower level of feed intake was designed to meet NRC requirements at thermoneutrality (NRC, 1978), while the higher intake of the C90 group was designed to approximate the usual increase in *ad libitum* intake during adaptation to a cold environment (Fuller, 1965). Calves were allowed to adapt to treatments for 21 days, during which time a pelleted alfalfa/barley ration (Table II-1; 168.8 g crude protein $kg^{-1}DM$, 16.31 MJ gross energy $kg^{-1}DM$) was fed every 2 hours from an automatic feeder in order to achieve a steady-state in nutrient absorption from the small intestine. The weight of any unconsumed feed was recorded each morning. Calves were weighed weekly and feed offered adjusted accordingly.

NITROGEN BALANCE TRIAL:

Starting at 49 days of age, total collection of urine and feces was undertaken for 7 consecutive days. A pooled sample of urine or feces comprised a 10 % fraction of daily samples. Bacterial growth in urine was inhibited by 100 mL of 6 N HCl; a 20 mL aliquot of urine was taken daily for N^{τ}-methylhistidine determination. Crude protein content of urine, feces, and feed was analyzed by the Kjeldahl method (AOAC, 1984).

Oxygen consumption of each calf was measured for an 8 hour period on the third or fourth day of the balance trial by indirect calorimetry in an open-circuit system as described by Young *et al.* (1975). Air was drawn through a box-like hood encompassing the calf's head; calves had been previously adapted to wearing the hood. During the measurement of oxygen consumption, feed was given every 2 hours through a port in the hood. Oxygen concentration in the incoming and outgoing air was continuously monitored by a dual-channel paramagnetic analyzer (Servomex Model OA184; Taylor Servomex, Crowborough, U.K.) and calibration of the respiratory gas exchange system was done according to the iron-burn procedure of Young *et al.* (1984). Metabolic rate was calculated using M^{c}Lean's equation (1972).

CONTINUOUS INFUSION PROCEDURE:

At 55 days of age, each calf was fitted with two jugular vein catheters, an infusion catheter in the right jugular vein (0.5 m long) and a sampling catheter in the left jugular vein (0.1 m long). At 56 days of age, each calf was given a continuous infusion of L-[ring-2,6-^{3}H]-phenylalanine (59 Ci mmol^{-1}, 40 μCi kg^{-1} BW; Amersham Corp., Oakville, Canada) in sterile physiological saline (9 g NaCl and 10 mmol phenylalanine L^{-1}) at a rate of 30 mL hour^{-1} (approximately 325 μCi hour^{-1}) for 8 hours via a peristaltic pump (Pharmacia Peristaltic Pump P-3 with 2.1 mm i.d. silicon tubing; Pharmacia Fine Chemicals, Uppsala, Sweden). Labeled phenylalanine was administered via a continuous infusion because a single flooding dose would have been prohibitively expensive for animals of the size used in the present experiment.

TISSUE AND BLOOD SAMPLING:

A 10 mL blood sample was taken prior to and every 20 minutes during the infusion. Samples were placed on ice in heparinized test tubes (140 USP units of heparin sodium; Allen and Hanburys, Toronto, Canada). Plasma was removed by centrifugation (569 x *g* for 15 minutes at 4°C) within 2 hours of sampling and stored at -70°C until analyzed.

At the conclusion of the infusion, each calf was anesthetized with thiopentone sodium (Intraval sodium, 1 mL/2.72 kg body weight; M.T.C. Pharmaceuticals, Mississauga, Canada) and killed by decapitation. Approximately 5 g of tissue from the rumen wall, rumen papillae, omasum, intestine (duodenum), kidney, liver, heart, longissimus dorsi, biceps femoris, and perineal skin were immediately removed, rinsed with cold sterile saline if necessary, and placed on ice. Samples were weighed, minced, and then homogenized in 10 mL ice-cold 2% (v/v) perchloric acid (Fisher Scientific Co., Fair Lawn, USA) with a Polytron homogenizer (Brinkman Instruments, Rexdale, Canada) to precipitate tissue proteins. The homogenizer blades were rinsed with an additional 5 mL (10 mL for the skin) of ice-cold 2% perchloric acid. The homogenate was centrifuged at 2280 x *g* to separate the protein pellet from the acid supernatant. The protein pellet was washed and re-centrifuged twice with 10 mL of ice-cold 2% perchloric acid to remove any free amino acids followed by lyophilization. Lyophilized pellets, homogenates, and infusate aliquots were frozen at -50°C until analyzed. The homogenate supernatant was assumed to represent the tissue intracellular phenylalanine fraction and the homogenate pellet, the tissue protein-bound phenylalanine fraction. Total organ and tissue weights were also recorded (see Appendix 2).

ANALYTICAL TECHNIQUES:

Specific Radioactivity of Phenylalanine in Tissues and Plasma:

To determine the specific radioactivity of phenylalanine, an enzymatic decarboxylation to ß-phenylethylamine followed by organic extraction was necessary to prevent contamination from labeled products such as tyrosine and tyramine. A modification of the method of Suzuki and Yagi (1976) as outlined by M^{c}Allister (1987) was used.

For analysis of the tissue protein fraction, approximately 25 mg of the freeze-dried pellet was hydrolyzed in 3 mL 6 N HCl for 24 hours at 110°C, dried in a vacuum centrifuge (Savant Speed-vac Concentrator Model SVC-200H; Savant Instruments Inc., Farmingdale, USA), and resuspended in 2 mL 0.5M sodium citrate.

Aliquots of plasma (2.5 mL) were deproteinized by addition of 0.7 mL ice-cold 1.5 N perchloric acid and centrifugation (3100 x *g* at 4°C for 15 minutes). Following neutralization of 2 mL of deproteinized plasma with 0.6 mL of saturated tripotassium citrate (BDH Chemicals LTD., Poole, U.K.), samples were centrifuged (3100 x *g* at 4°C for 15 minutes) to remove precipitated potassium perchlorate. This neutralized deproteinized plasma was used for enzymatic conversion and organic extraction. Intracellular samples (2 mL) were also neutralized by addition of saturated tripotassium citrate (0.5 mL) and treated in the same way as deproteinized neutralized plasma.

Radioactivity of ß-phenylethylamine in the final extract was determined after an aliquot was mixed with liquid scintillation fluid (Beckman Ready-gel; Beckman Instruments [Canada] Ltd., Mississauga, Canada), dark adapted, and counted in a liquid scintillation spectrometer (Mark III Liquid Scintillation System Model 6880; Searle Analytic Inc., Des Plaines, USA) until 10,000 counts had accumulated. The counting efficiency was 0.33-0.40 for ^{3}H using the external standard pulse height method in the variable window mode.

The concentration of ß-phenylethylamine in the final extract was analysed using high performance liquid chromatography (HPLC) according to the method outlined by M^{c}Allister (1987). Ethanolamine (Fisher Scientific Co., Fair Lawn, USA) was used as an internal standard, and both standards and samples were derivatized with *o*-phthaldialdehyde (Sigma Chemical Co., St. Louis, USA) according to the method of Jones and Gilligan (1983).

Determination of Free and Protein-bound Phenylalanine in Tissues:

The concentration of free phenylalanine in the intracellular fraction or bound in the protein fraction was determined by HPLC analysis following neutralization of 0.5 mL of the tissue homogenate supernatant with 0.5 mL saturated potassium borate and hydrolysis of 20 mg of the protein pellet in 3 mL of 6 N HCl, respectively. Samples were derivatized and analyzed as described previously for ß-phenylethylamine. For the heart, longissumus dorsi, and biceps femoris muscles, a value for R, the ratio of protein-bound to intracellular free phenylalanine concentrations, was calculated.

Determination of Urinary N$^{\tau}$-Methylhistidine:

Urine was analyzed for N$^{\tau}$-methylhistidine by an HPLC method as described by Nakamura and Pisano (1976) and Wassner *et al.* (1980) with the following modifications. To 0.2 mL of urine or standards (5 x 10^{-4} M N$^{\tau}$-methylhistidine), 0.2 mL of internal standard (5 x 10^{-4} M histidinol) was added. Samples were deproteinized with 0.2 mL of 3.0 M $HClO_4$ and centrifuged at 2280 x *g* at 4°C for 15 minutes. A 0.1 mL aliquot of deproteinized urine was mixed in a screw-cap tube with 0.4 mL deionized water, 0.04 mL of 1.5 M NaOH, and 0.4 mL of 0.2 M $Na_2B_4O_7$ (pH 9.0). As they were vortexed, samples were derivatized with 0.25 mL of a fluorescamine solution (160 mg fluorescamine 100 mL^{-1} acetonitrile; Sigma Chemical Co., St. Louis, USA and Fisher Scientific Co., Fair Lawn, USA , respectively). After sitting for a few seconds to consume excess fluorescamine, 0.4 mL of 2.0 M HCl was added to samples. Tubes were mixed, capped, and incubated in a 90°C water bath for 45 minutes. Following two extractions with 1.5 mL diethyl ether (Fisher Scientific Co., Fair Lawn, USA) to remove a contaminant that co-eluted with N^{τ}-methylhistidine, samples were eluted from a 3 micron reverse phase C18 column (Supleco, Inc., Bellefonte, USA) using a binary gradient; solvent A was 8 mM ammonium acetate buffer (pH 5.0) and solvent B was acetonitrile. The gradient rose from 0.25 to 0.60 acetonitrile over 8.5 minutes at a solvent flow rate of 1.1 mL per minute.

Protein and RNA Content of Tissue:

Protein content of the freeze-dried pellets was determined according to the method of Hartree (1972) with bovine serum albumin as the standard. Absorbance of samples and standards at 650 nm was determined with a Spectronic 21 spectrophotometer (Milton Roy Co., Rochester, USA).

RNA content of freeze-dried pellets was determined according to a modification of the method of Munro and Fleck (1969). Approximately 25 mg of the freeze-dried pellet rather than fresh tissue were solubilized in 4 mL of 0.3 N potassium hydroxide during a 1 hour incubation at 37°C. A standard solution of calf liver RNA (Sigma Chemical Co., St. Louis, USA), used to construct a standard curve, was treated in the same manner as samples. Absorbance of standards and samples at 260 nm was determined in a dual-beam ultraviolet spectrophotometer (Unicam SP1800 Ultraviolet Spectrophotometer; Pye-Unicam LTD., Cambridge, U.K.) and was corrected for the presence of acid-soluble peptides by subtracting 0.001 O.D. units for each 1 μg protein per mL of protein in the sample. Protein concentration was determined according to the Bradford method (1976) as modified by Read and Northcote (1981), using bovine serum albumin as the standard.

The protein and RNA contents of the freeze-dried pellet were multiplied by the weight of the fresh tissue sample which was homogenized to give that pellet. The protein and RNA contents of the fresh tissue sample were in turn multiplied by the total organ or tissue weight in order the give the organ or tissue contents of protein and RNA, respectively.

CALCULATIONS:

Plasma Phenylalanine Flux:

The specific radioactivity (d.p.m. mmol^{-1}) of phenylalanine in plasma was plotted against sampling time for each animal, and the rise to plateau specific activity was fitted to a single exponential function using a nonlinear iterative procedure (SAS, 1985). The equation used was:

$$S_p = S_p\text{max} \cdot (1 - e^{-\lambda p.t}) \qquad (1)$$

where S_p is the plasma phenylalanine specific radioactivity at any time, $S_p$max is the plateau specific radioactivity, λ_p is the rate-constant (days^{-1}), and t is time (days) (Shipley and Clark, 1972). Phenylalanine flux (mmol day^{-1}) was calculated from the formula:

$$I = F \cdot S_p\max \qquad (2)$$

where I is the isotope infusion rate (μCi day^{-1}), F is the flux (mmol day^{-1}) and $S_p$max is the plateau specific radioactivity of phenylalanine in plasma (μCi mmol^{-1}).

Fractional Synthesis Rate of Protein in Tissues:

The fractional synthesis rate of protein in tissues was calculated according to modifications by Garlick *et al*. (1973) of the method of Waterlow and Stephen (1968) according to the formula:

$$\frac{S_B}{S_i} = \left(\frac{\lambda_i}{\lambda_i - k_s}\right)\left(\frac{1 - e^{-k_s.t}}{1 - e^{-\lambda_i.t}}\right) - \left(\frac{k_s}{\lambda_i - k_s}\right) \qquad (3)$$

where S_B and S_i are the specific radioactivities (μCi mmol^{-1}) of protein-bound and intracellular free phenylalanine, respectively, k_s (% day^{-1}) is the fractional synthesis rate of protein in a tissue, t is the length of the infusion in days, and λ_i is the rate constant describing the rise to plateau of the specific radioactivity of free phenylalanine in the tissue.

The value of λ_i was not determined directly due to technical difficulties. Instead, the approximations of Garlick *et al*. (1973) were used. For all tissues except the cardiac and skeletal muscles, individual values of λ_p obtained from equation (1) were substituted for λ_i. In muscle, which has a slower turnover rate, the appropriate term for λ_i is $R \cdot k_s$, where R is the mean concentration ratio, bound:free phenylalanine in each tissue.

In calculating the fractional synthesis rate of protein in tissues and organs, the intracellular free phenylalanine pool was assumed to represent the true precursor pool, phenylalanyl-tRNA, so calculated fractional synthesis rates are maximum estimates of true rates (Davis *et al.*, 1981).

Absolute Synthesis Rate of Protein in Tissues:

The absolute synthesis rate of protein (g day^{-1}) was calculated as the product of protein content (g protein) and the fractional synthesis rate of protein in each tissue (% day^{-1}). In addition, the total amount of skeletal muscle in the body was assumed to be 0.365 of body weight from studies of calf body composition by Ugarte *et al.* (1974). For each calf, the product of the average protein content and the fractional synthesis rate of protein in the longissimus dorsi and biceps femoris muscles was used as an estimate of the absolute synthesis rate of protein in skeletal muscle.

Whole-body Protein Synthesis and Degradation and the Energy Cost of Whole-body Protein Synthesis:

Whole-body protein synthesis (g day^{-1}) was calculated as the sum of the absolute synthesis rates of protein in all tissues sampled. Whole-body protein degradation was calculated as the difference between synthesis as determined above and protein gain from nitrogen retention (g protein = g N $\cdot$ 6.25). The energy cost of protein synthesis was assumed to be 4.5 kJ g^{-1} (Webster, 1976), which is a minimum estimate based on the theoretical use of 5 mol ATP for each peptide bond.

Ribosomal Protein Synthetic Activity:

Intensity of ribosomal protein synthesis in a tissue, or the rate of protein synthesis per unit of RNA, was calculated by dividing the absolute synthesis rate of protein by total cellular RNA.

Muscle Protein Metabolism:

Muscle protein synthesis was determined as described for the absolute synthesis rate of protein. Muscle protein degradation was calculated as the average daily urinary N^{τ}-methylhistidine excretion (μmol day^{-1} kg^{-1} BW) divided by the mean muscle protein content of N^{τ}-methylhistidine in cattle (3.5 μmol g^{-1} muscle protein; Nishizawa *et al.*, 1979). Muscle protein gain was calculated as the difference between synthesis and degradation.

STATISTICS:

All results were analyzed by one-way analysis of variance and SNK multiple comparison of means as described by Steele and Torrie (1980) using SPSS-X (SPSS, 1988). Linear and non-linear regressions were performed using SAS (SAS, 1985). The fractional synthesis rates of protein in the rumen wall and rumen papillae within each treatment were compared with a t-test (Steele and Torrie, 1980) using SAS (SAS, 1985).

RESULTS

Calf Parameters:

Means for body weight, feed intake, gain, ratio of gain to feed intake, heat production and protein retention of calves are found in Table II-2. As designed, the average daily intake of the calves in the C90 group was about 20% greater than either of the groups at the lower level of intake. Compared with the W72 group, the average daily gain was reduced in the C72 group ($P<0.05$), but not the C90 group. Retarded body weight gain in the cold led to a significant reduction in the efficiency of use of feed for gain ($P<0.05$).

Although heat production tended to be elevated in the cold, there were no significant differences between treatments. Whole-body retention of crude protein was depressed in the C72 group ($P<0.05$) compared with the other two groups, but in the C90 group it was maintained at the same level as the W72 group.

Intravenous Infusion of ^{3}H-Phenylalanine:

Three to four calves in each group were infused with ^{3}H-phenylalanine for 8 hours and plasma phenylalanine specific radioactivity at each sampling time after beginning the infusion was averaged by group (Figure II-1). Plasma phenylalanine specific radioactivity usually plateaued within 1.5 hours and rate constants ranged from 40 to 94 day^{-1}, averaging 53 day^{-1}. Although there was no effect of environment on the rate constant, with means of 43, 55, and 63 day^{-1} for the W72, C72, and C90 groups, respectively, plateau plasma specific radioactivity was significantly higher in the W72 group compared with both of the groups in the cold ($P<0.05$).

Phenylalanine specific radioactivity in the intracellular pool was always lower than that of the plasma pool. The ratio of intracellular:plasma specific radioactivity ranged from 0.37-0.82 and tended to be lowest in gastrointestinal tract tissues and highest in muscles (Appendix 3).

Fractional Synthesis Rate of Protein in Tissues:

Fractional synthesis rates of protein in ten tissues and organs are shown in Figures II-2A to II-2E. Fractional synthesis rates of protein in both skeletal muscles were reduced by about 50% in the C72 group ($P<0.05$) compared with either of the two other groups, while in the C90 group they were maintained at the same level as the W72 group. A similar trend to that observed in skeletal muscle was seen in the fractional synthesis rates of protein in the kidney ($P<0.05$) and the skin ($P=0.13$). The fractional synthesis rate of protein was most rapid in the gastrointestinal tract but was not significantly affected by environmental temperature.

Absolute Synthesis Rate of Protein in Tissues:

Absolute synthesis rates of protein (Table II-3) in the two individual skeletal muscles and in the total skeletal musculature were depressed in the C72 group compared with the W72 group ($P<0.05$), but not in the C90 group. A similar but non-significant trend was seen in the skin ($P<0.10$). As with the fractional synthesis rates, absolute synthesis rates of protein in gastrointestinal tissues were highly variable and not affected by temperature.

Protein Synthesis per Unit RNA:

Environmental temperature had no significant effect on protein synthetic activity per unit of RNA (Table II-4), although there was a trend for the rate in biceps femoris to be slower in the C72 group compared with the control group (P<0.07).

Whole-body Protein Synthesis, Gain, and Degradation, Energy Costs of Protein Synthesis, and Phenylalanine Flux:

Estimates of whole-body protein synthesis, gain, and degradation, as well as of the energy cost of protein synthesis are presented in Table II-5 together with phenylalanine flux. Environmental temperature had no effect on whole-body protein synthesis. There was a non-significant trend for whole-body protein gain on a body weight basis to be about 40% lower in the C72 group compared with the W72 group; no such decrease was seen in the C90 group. Whole-body protein degradation, calculated as the difference between whole-body protein gain and whole-body protein synthesis, was not affected by environment. Similarly, the energy cost of whole-body protein synthesis estimated from the sum of protein synthesis in all tissues was not influenced by environment. Phenylalanine flux, or the irreversible loss of phenylalanine from the plasma per day, was elevated in both groups in the cold (P<0.01).

Muscle Protein Turnover:

Muscle protein synthesis (Table II-6), estimated from the absolute synthesis rate of protein in all muscle in the body, was decreased by about 40% in the C72 group (P<0.05), but not in the C90 group. Muscle protein degradation, estimated from N^{τ}-methylhistidine excretion, tended to be elevated in both groups in the cold (P<0.10). Muscle protein gain, calculated as the difference between muscle protein degradation and synthesis, followed the same pattern among treatments as the one shown by protein synthesis, with gain in the C72 group significantly lower than both the W72 and C90 groups ($P<0.01$), but no difference between the latter two groups.

DISCUSSION

Animal Performance and Nitrogen Balance:

Depressed growth in both groups of calves in the cold (Table II-2) is similar to findings reported by Ames and co-workers for lambs and feedlot steers (Ames *et al.*, 1980). Additional feed intake permitted the average daily gain of the C90 group to be intermediate to the other two groups, while in the C72 group feed intake was not sufficient to prevent decreased gain. Because additional feed was required to maintain gain in the C90 group and because gain decreased in the C72 group, feed conversion efficiency dropped in both. Pigs at 10°C fed *ad libitum* showed similar reductions in efficiency (Fuller, 1965).

In the cold-adapted calves, heat production was 20 % higher than in calves in the warm environment. This increase was not significant, likely due to the fact that heat production was only measured on one occasion. Webster *et al.* (1978) also observed an increase in heat production in dairy-type calves adapted to 5°C compared with calves adapted to 15°C. It is probable that in the cold-adapted calves in the present experiment, metabolizable energy was diverted away from tissue growth toward heat production.

Reduced whole-body protein retention in the C72 group but not in the C90 group indicates that a 20 % increase in feed intake permitted the latter to maintain a similar level of lean tissue gain as the W72 group. It is probable that in the C72 group more amino acids were oxidised as an energy source rather than used for protein synthesis, thus reducing protein retention (Lindsay *et al.*, 1988). This is supported by a significant increase in urinary nitrogen excretion in the

C72 group but not in the C90 group compared with the W72 group ($P<0.03$, Appendix 1).

Fractional Synthesis Rate of Protein in Tissues:

The continuous infusion method used in this experiment to calculate the fractional synthesis rate of protein incorporates assumptions discussed in detail by Garlick *et al*. (1973). One assumption is that protein synthesis and degradation are equal to each other, with no protein gain occurring (Waterlow *et al*., 1978). Obviously, in the present experiment this is not the case, since all calves exhibited protein gain. However, animals were fed every 2 hours in an attempt to encourage a steady-state in phenylalanine absorption from the gastrointestinal tract and to minimize increases in muscle protein synthesis after feeding (Garlick *et al*., 1973).

A further assumption involves the choice of whether the intracellular or plasma pool of phenylalanine better represents the true precursor for protein synthesis, phenylalanyl-tRNA. Intracellular specific radioactivity of a tracer amino acid will always be lower than that of the plasma pool due to dilution of the label from unlabeled amino acids released by proteolysis (Rannels *et al*., 1982; see Appendix 3). Therefore, fractional synthesis rates of protein calculated using the intracellular or plasma phenylalanine pool to represent the precursor pool are only maximum and minimum estimates, respectively, of the true value. This inequality resulted in significant differences between the maximum and minimum estimates of the fractional synthesis rate of protein in all tissues ($P<0.0002$ for rumen wall, $P<0.0157$ for intestine, $P<0.0001$ for all others). However, in the present experiment, use of minimum estimates did not alter interpretation of the effect of environmental temperature on fractional synthesis rates, except that for the C90 group the minimum fractional synthesis rate of protein in the biceps femoris muscle was intermediate to and significantly different from rates in both the W72 and C72 groups, and for the kidney there was no longer a significant effect of temperature (Appendix 5).

Gastrointestinal tissues exhibited the highest and muscles the lowest fractional synthesis rates of protein (Figures II-2A to II-2E), and variablity appeared to be directly related to fractional synthesis rates. Some variability in gastrointestinal tissue rates may be due to intracellular recycling of label during an 8 hour-long infusion when protein turnover is rapid (Waterlow *et al.*, 1978) or dilution of the intracellular phenylalanine pool with unlabeled phenylalanine from protein degradation after the infusion is terminated. Recently, Attaix and Arnal (1987) have used a flooding dose of ^{3}H-valine to measure the fractional synthesis rate of protein in gastrointestinal tissues of lambs. Since tissues were sampled within 30 minutes of isotope injection, recycling of label and thus variability in fractional synthesis rates of protein were reduced. The actual fractional synthesis rates of protein were similar to those in the present experiment.

Mean fractional synthesis rates of protein in rumen papillae were not significantly higher than in the rumen wall. It was expected that they would be higher, since the cells of the papillae are sloughed off and need to be replaced frequently (Davis *et al.*, 1981).

The fractional synthesis rate of protein in intestine was slightly higher than the rate reported for heifers by Lobley *et al.* (1980), while the value for the rumen wall was similar to that reported for 273 kg steers by M^{c}Bride *et al.* (1989). Major organs such as the kidney, liver, and heart exhibited fractional synthesis rates of protein approximately triple those reported for steers by M^{c}Bride *et al.* (1989); discrepancies may be attributed to differences in age between experimental animals. Rates for large protein depots, such as muscle and skin, were similar to those reported for heifers by Lobley *et al.* (1980).

Reduced fractional synthesis rates of protein in muscle in the C72 group agree with findings of M^{c}Allister (1987) that the fractional synthesis rate of protein in soleus muscle was decreased in rats acclimated to 4°C, despite increased food intake. Environmental temperature did not affect the fractional synthesis rate of protein in muscle from pigs fed at either a low or high level of intake (Lindsay *et al.*, 1988). However, their results were confounded by the fact that feed intake per unit of body weight was actually higher for both groups of animals at 10°C than those at 35°C. Thus, feed intake was probably more than adequate to supply demands of amino acids for energy and protein synthesis, even in the pigs on the low level of intake at 10°C. The fractional synthesis rate of protein in the skin tended to mimic the pattern observed in muscle (P-0.13), but difficulties in sampling and grinding the skin elevated variability.

Depressed rates of protein synthesis in muscle and skin along with reduced nitrogen retention in the C72 group suggest that feed intake was inadequate to support lean tissue growth at the same rate as in the other two groups. The fact that protein synthesis was decreased in muscle and skin rather than in all tissues implies that those two tissues have a lower priority for limited nutrients than others (Buttery and Bryan, 1986). Allison and Wannemacher (1965) contended that during conditions of nutritional stress, skin would serve as a significant labile protein reserve along with muscle, resulting in curtailed protein synthesis. This is supported by results showing that protein synthesis in skin and muscle were reduced by 26 % and 66 %, respectively, following a two-day fast (Preedy *et al.*, 1983). Therefore, skin protein may be mobilized along with muscle protein in order to meet amino acid requirements in situations where feed intake is inadequate. Further studies on the effect of feed intake and cold-adaptation on skin protein degradation and amino acid release are necessary to confirm this hypothesis.

An effect of adaptation to a cold environment on the fractional synthesis rate of protein in the kidney has not been reported before. As a site of gluconeogenesis, carbon skeletons of amino acids in the kidney were likely used as a source of glucose (Buttery and Annison, 1973) rather than for protein synthesis.

Lack of response in liver protein synthesis to cold-adaptation in calves is supported by similar results in chicks fed *ad libitum* at 22°C compared with 30°C (Aoyagi *et al.*, 1988) and rats fed *ad libitum* at 5°C compared with 25°C (M^{c}Allister, 1987). In the present experiment, the fractional synthesis rate of protein in the heart was also unchanged after acclimation to a cold environment, although M^{c}Allister (1987) observed that protein synthesis in the heart was elevated in rats exposed to 5°C for 20 days compared with 25°C.

None of the gastrointestinal tissues exhibited a response in fractional synthesis rates of protein to cold-adaptation. Some alteration might have been expected, since turnover time in enterocytes from piglets adapted to 10°C was slower compared with 35°C (Dauncey *et al.*, 1983).

Absolute Synthesis Rate of Protein in Tissues:

Absolute synthesis rates of protein (Table II-3) were mainly influenced by changes in fractional synthesis rates as protein content of tissues did not vary with treatment (Appendix 6). However, unlike the fractional synthesis rate of protein, there was no significant effect of temperature on the absolute synthesis rate of protein in kidney.

Protein Synthesis per Unit RNA:

The rate of protein synthesis per unit RNA, also termed RNA activity (Table II-4) is an indication of the rate at which translation of mRNA is occuring at the level of the ribosome (Millward *et al.*, 1976). Due to high variability in tissue RNA content or capacity for protein synthesis (Appendix 7), there were no significant effects of environment on RNA activity. Variability may have been due to actions of RNAases after tissues were sampled.

Whole-body Protein Synthesis, Gain, and Degradation, Energy Costs of Protein Synthesis, and Phenylalanine Flux:

Values for whole-body protein synthesis (Table II-5) estimated from the sum of the absolute synthesis rates of protein in all tissues were similar to results reported for 250 kg heifers using a constant infusion of tyrosine (Lobley *et al.*, 1980). In the present experiment, whole-body protein synthesis was not influenced by environmental temperature. Lindsay *et al.* (1988) reported that in pigs whole-body protein synthesis calculated from tyrosine flux corrected for oxidation was not influenced by environmental temperature, while in chicks acclimated to 22 °C, whole-body protein synthesis measured using a flooding dose of ^{3}H-phenylalanine was elevated compared with 30°C (Aoyagi *et al.*, 1988).

Protein degradation (Table II-5), estimated as the difference between whole-body protein synthesis and protein gain, was not significantly affected by environment, mainly due to high variability in estimates of whole-body protein synthesis. Although there are energy costs related to protein degradation as well as synthesis, quantitative estimates are ill-defined.

Protein synthesis is an energy-requiring process, using approximately 4.5 kJ per g protein synthesized (Webster, 1976), and accounting for about 20 % of heat production (Webster, 1981). In the present experiment, at 4.5 kJ g^{-1} protein synthesized, whole-body protein synthesis was 18.5, 11.5, and 16.7 % of daily heat production in the W72, C72, and C90 groups, respectively. Although protein synthesis appeared to account for a lower proportion of heat production in the C72 group, it was not significantly different.

Heat production was linearly related to the rate of whole-body protein synthesis in pigs (Reeds *et al.*, 1980). Although there tended to be a similar relationship between those parameters in the data from the two groups of calves in the cold, it was not significant. Since there was no significant relationship between protein synthesis and heat production nor any change in the proportion of heat production due to protein synthesis, it is unlikely that significant changes in protein synthesis contributed to cold thermogenesis in the present experiment.

It was not possible to determine whether the elevation in phenylalanine flux in both groups of calves in the cold was due to an increased rate of protein synthesis or oxidation. Since protein synthesis in a variety of tissues was either unchanged or decreased in the cold-adapted calves, it is unlikely that a greater proportion of phenylalanine flux was used for protein synthesis. On the other hand, the observed increase in heat production in the cold-adapted calves could result from accelerated oxidation of energy substrates, including amino acids. This is supported by results demonstrating an elevated tyrosine catabolic rate in pigs acclimated to 10°C compared with 35°C (Lindsay *et al.*, 1988). Higher phenylalanine oxidation in the cold-adapted calves in the present experiment could also account for lower mean values of $S_p$max in the C72 and C90 groups. Unfortunately, the fraction of phenylalanine flux going to oxidation in the present experiment is unknown.

Muscle Protein Turnover:

It appears that the effect of acclimation to a cold environment on protein synthesis in muscle can be overcome by higher feed intake. Since the same diet was fed to all groups, protein intake increased along with feed intake, as did the absolute synthesis rate of protein in all muscle and in skin (Figure II-3). Garlick and Lobley (1987) presented evidence that in warm-acclimated lambs fed from 0.5 to 1.5 times maintenance the fractional synthesis rate of protein in the hind limb increased and suggested that muscle is sensitive to increments in nutrient intake. Skin protein synthesis may also be sensitive to nutrient intake.

Muscle protein degradation can be estimated from urinary N^{τ}-methylhistidine excretion subsequent to myofibrillar protein breakdown. Although not all N^{τ}-methylhistidine comes from muscle, Nishizawa (1979) calculated that it contributed more than 93 % of the N^{τ}-methylhistidine contained in cattle tissues. N^{τ}-methylhistidine excretion in the present experiment was about 80 % lower than previously reported for restricted-fed calves of the same breed and age (Thompson *et al.*, 1987) and lower than predicted values from the regression equation of Harris and Milne (1981). Higher feed intakes in the aforementioned experiments compared with the present experiment (1.7-2.0 times higher: Thompson *et al.*, 1987; *ad libitum*: Harris and Milne, 1981) may have increased the degradation of actin and myosin since protein degradation usually increases along with feed intake (Reeds and Fuller, 1983).

Cold-adaptation elevated N^{τ}-methylhistidine excretion by about 30 %, which is an increment similar to that reported by Thompson *et al.* (1987) for restricted-fed calves acutely exposed to -10°C. The trend toward increased muscle protein degradation in cold-adapted calves was supported by reports from Brown *et al.* (1984) that muscle protein degradation was elevated in *ad libitum*-fed rats exposed to 4°C. More rapid protein degradation in the cold would likely provide amino acid carbon skeletons for oxidation and gluconeogenesis, as discussed previously.

Depressed muscle protein synthesis combined with a trend toward accelerated muscle protein degradation resulted in reduced muscle protein gain in the C72 group compared with the W72 and C90 groups.

Muscle protein deposition efficiency, indicated by the ratio protein gain:protein synthesized (Reeds *et al.*, 1980), was 0.614, 0.091, and 0.495 in the W72, C72, and C90 groups, respectively. It is apparent that protein deposition in the C72 group was much less efficient than in the other two groups.

Conclusion:

Protein depots such as muscle and skin demonstrated the most marked alterations in protein turnover following cold-adaptation. Substrate availability seems to play a major role in the response to a cold environment, as additional feed intake in the cold usually alleviated observed changes. Additional information on oxidation and use of phenylalanine for gluconeogenesis in cold-adapted calves would clarify the observed effects of a cold environment on phenylalanine flux. Although it seems unlikely that increased protein synthesis contributes to cold-induced thermogenesis, the role of protein degradation could be delineated by more accurate estimates of whole-body protein degradation.

TABLE II-1: Composition of diet.

COMPONENT	CONCENTRATION (g kg^{-1} DM)
Barley Grain	566
Dehydrated Alfalfa	208
Dehydrated Beet Pulp	102
Soybean Meal	69.6
Molasses	17.4
$CaPO_4$	5.4
$CaCO_3$	5.6
NaCl	5.6
Vitamin ADE Premix	5.6
"PERMAPEL" Pelleting Agent	16.8
Crude Protein	168.8
Gross Energy (MJ kg^{-1} DM)	16.3

TABLE II-2: Average body weight, average daily intake, average daily gain, gain/feed (% efficiency), heat production and crude protein retention of calves adapted to two environmental temperatures (Mean ± SEM).

PARAMETER	W72	C72	C90	P VALUE
n	4	4	3	
Environmental Temperature (°C)	20.3	-3.3	-3.3	N/A
Body Weight (kg)	68.5 ± 3.4	61.3 ± 3.3	72.3 ± 5.9	0.213
Average Daily Intake (kg day^{-1})	1.40^{a} ± 0.03	1.38^{a} ± 0.06	1.70^{b} ± 0.09	0.011
Average Daily Gain (kg day^{-1})	0.610^{a} ± 0.044	0.261^{b} ± 0.101	0.451^{a,b} ± 0.052	0.025
Gain/Feed (% Efficiency)	43.5^{a} ± 3.0	18.9^{b} ± 7.0	26.4^{b} ± 2.2	0.018
Heat Production (kJ day^{-1} kg^{-1} BW)	205 ± 19	254 ± 14	241 ± 32	0.249
Protein Retained (g Crude Protein day^{-1})	113^{a} ± 5.1	60.0^{b} ± 11	113^{a} ± 26	0.040

N/A: Not applicable.
^{a,b}: Means in the same row followed by different letters are significantly different ($P<0.05$).

TABLE II-3: Absolute synthesis rate of protein* (g protein day^{-1}) in tissues from calves adapted to two environmental temperatures (Mean ± SEM).

PARAMETER	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	1.96^{a} ± 0.34	0.80^{b} ± 0.20	2.38^{a} ± 0.55	0.029
Biceps Femoris	2.97^{a} ± 0.18	1.14^{b} ± 0.21	2.67^{a} ± 0.48	0.003
All Muscle†	96.5^{a} ± 8.4	49.2^{b} ± 9.3	102.3^{a} ± 11.5	0.008
Skin	75.0 ± 13.6	32.1 ± 7.8	62.6 ± 12.6	0.064
Kidney	3.94 ± 0.54	4.92 ± 0.40	5.61 ± 0.68	0.151
Liver	50.0 ± 2.8	54.3 ± 7.1	41.0 ± 13.1	0.521
Heart	3.62 ± 0.34	4.33 ± 0.37	5.50 ± 0.93	0.110
Rumen Wall	61.4 ± 16.9	67.8 ± 4.2	87.1 ± 22.4	0.520
Omasum	17.4 ± 2.7	15.2 ± 3.2	22.4 ± 6.7	0.495
Intestine	291.2 ± 143.1	173.4 ± 43.9	317.7 ± 207.8	0.728

---

*: Calculated as described in Materials and Methods; fractional synthesis rate of protein calculated assuming intracellular phenylalanine is the precursor pool.

†: Whole body content of muscle estimated as 0.365 of body weight (Ugarte *et al.*, 1974); the fractional synthesis rate of protein was the average of the two muscles sampled, assuming intracellular phenylalanine as the precursor pool.

[a,b]: Means in the same row followed by different letters are significantly different (P<0.05).

TABLE II-4: Protein synthetic activity of RNA* (mg protein synthesized day^{-1} mg^{-1} RNA) in tissues from calves adapted to two environmental temperatures (Mean ± SEM).

PARAMETER	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	3.36 ± 0.67	1.38 ± 0.67	2.98 ± 0.77	0.151
Biceps Femoris	3.17 ± 0.46	1.70 ± 0.46	3.48 ± 0.53	0.063
Skin	3.94 ± 0.75	2.05 ± 0.75	4.50 ± 0.87	0.128
Kidney	3.82 ± 0.37	3.55 ± 0.37	4.81 ± 0.42	0.125
Liver	6.18 ± 0.44	5.44 ± 0.44	5.46 ± 0.51	0.447
Heart	2.98 ± 0.19	2.88 ± 0.19	3.41 ± 0.22	0.217
Rumen Wall	6.92 ± 1.03	6.96 ± 1.03	8.05 ± 1.19	0.738
Rumen Papillae	8.16 ± 1.00	7.83 ± 1.00	10.37 ± 1.16	0.270
Omasum	9.19 ± 1.38	9.08 ± 1.38	9.19 ± 1.59	0.669
Intestine	12.08 ± 4.41	8.54 ± 4.41	8.82 ± 5.09	0.828

*: Calculated as described in Materials and Methods; fractional synthesis rate calculated assuming intracellular phenylalanine is the precursor pool.

TABLE II-5: Estimation of whole-body protein synthesis, whole-body protein gain, whole-body protein degradation, and average phenylalanine flux kg^{-1} body weight of calves adapted to two environmental temperatures (Mean ± SEM).

PARAMETER	W72	C72	C90	P VALUE
n	4	4	3	
WBPS[†] From Sum of ASR (g day^{-1} kg^{-1} BW)	8.47 ± 1.86	6.46 ± 0.55	9.45 ± 3.36	0.576
Whole-body Protein Gain (g day^{-1} kg^{-1} BW)	1.65 ± 0.05	0.98 ± 0.18	1.60 ± 0.40	0.103
Whole-body Protein Degradation (g day^{-1} kg^{-1} BW)	6.82 ± 1.88	5.48 ± 0.62	7.85 ± 3.12	0.699
Energy Cost of WBPS from sum of ASR[¶] (kJ kg^{-1} BW)	38.1 ± 8.4	29.1 ± 2.5	42.5 ± 15.1	0.576
Phenylalanine Flux (mmol day^{-1} kg^{-1} BW)	2.48[a] ± 0.05	2.82[b] ± 0.11	3.02[b] ± 0.10	0.009

[†]: WBPS, Whole-body protein synthesis.

: ASR, Absolute synthesis rate.

[¶]: Assuming the energy cost of protein synthesis is 4.5 kJ g^{-1} (Webster, 1976).

[a,b]: Means in the same row followed by different letters are significantly different ($P<0.05$).

TABLE II-6: Estimation of muscle protein synthesis, muscle protein degradation, and muscle protein gain kg^{-1} body weight of calves adapted to two environmental temperatures (Mean ± SEM).

PARAMETER	W72	C72	C90	P VALUE
n	4	4	3	
Muscle Protein Synthesis* (g day^{-1} kg^{-1} BW)	1.404^{a} ± 0.082	0.807^{b} ± 0.160	1.418^{a} ± 0.125	0.014
Muscle Protein Degradation† (g day^{-1} kg^{-1} BW)	0.543 ± 0.036	0.734 ± 0.083	0.715 ± 0.042	0.098
Muscle Protein Gain (g day^{-1} kg^{-1} BW)	0.862^{a} ± 0.103	0.073^{b} ± 0.077	0.702^{a} ± 0.151	0.002

*: Whole body content of muscle estimated as 0.365 of body weight (Ugarte *et al.*, 1974); the fractional synthesis rate of protein was the average of the two muscles sampled, assuming intracellular phenylalanine as the precursor pool.

†: Calculated as daily urinary N^{τ}-methylhistidine excretion (μmol day^{-1} kg^{-1} BW) divided by the mean muscle protein content of N^{τ}-methylhistidine in cattle (3.5 μmol g^{-1} muscle protein; Nishizawa *et al.*, 1979).

: Calculated as the difference between muscle protein degradation and muscle protein synthesis.

^{a,b}: Means in the same row followed by different letters are significantly different (P<0.05).

FIGURE II-1: The rise in plasma phenylalanine specific radioactivity during an 8 hour infusion of ^{3}H-phenylalanine. Points are means for 3 to 4 calves in each group.

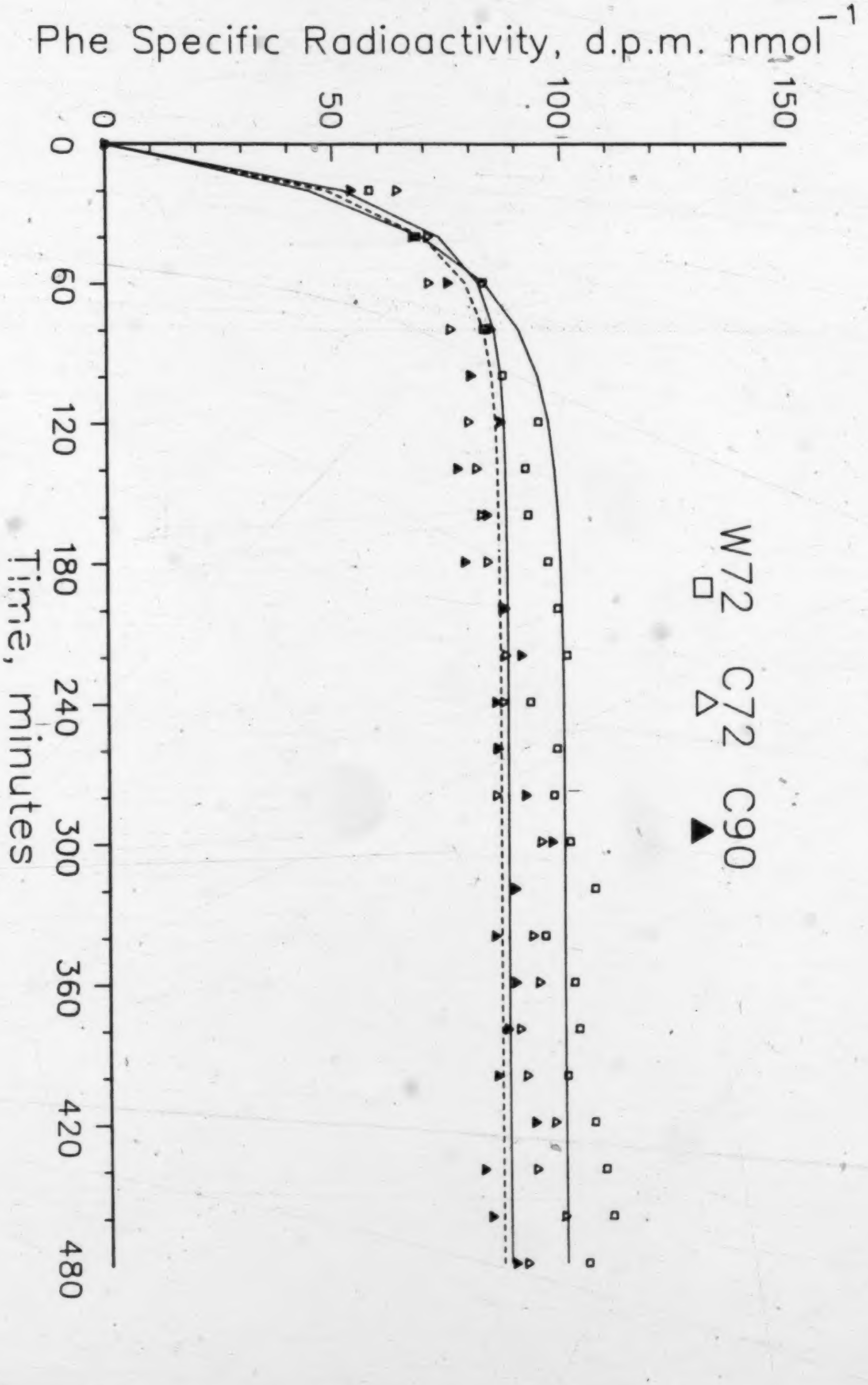

Phe Specific Radioactivity, d.p.m. nmol^{-1}
0
50
100
150
Time, minutes
0
60
120
180
240
300
360
420
480
W72 □
C72 △
C90 ▲

FIGURE II-2A: The fractional synthesis rate (% day^{-1}) of protein in muscle from calves adapted to two environmental temperatures. Values are means of 3 to 4 animals in each group and bars indicate the SEM.

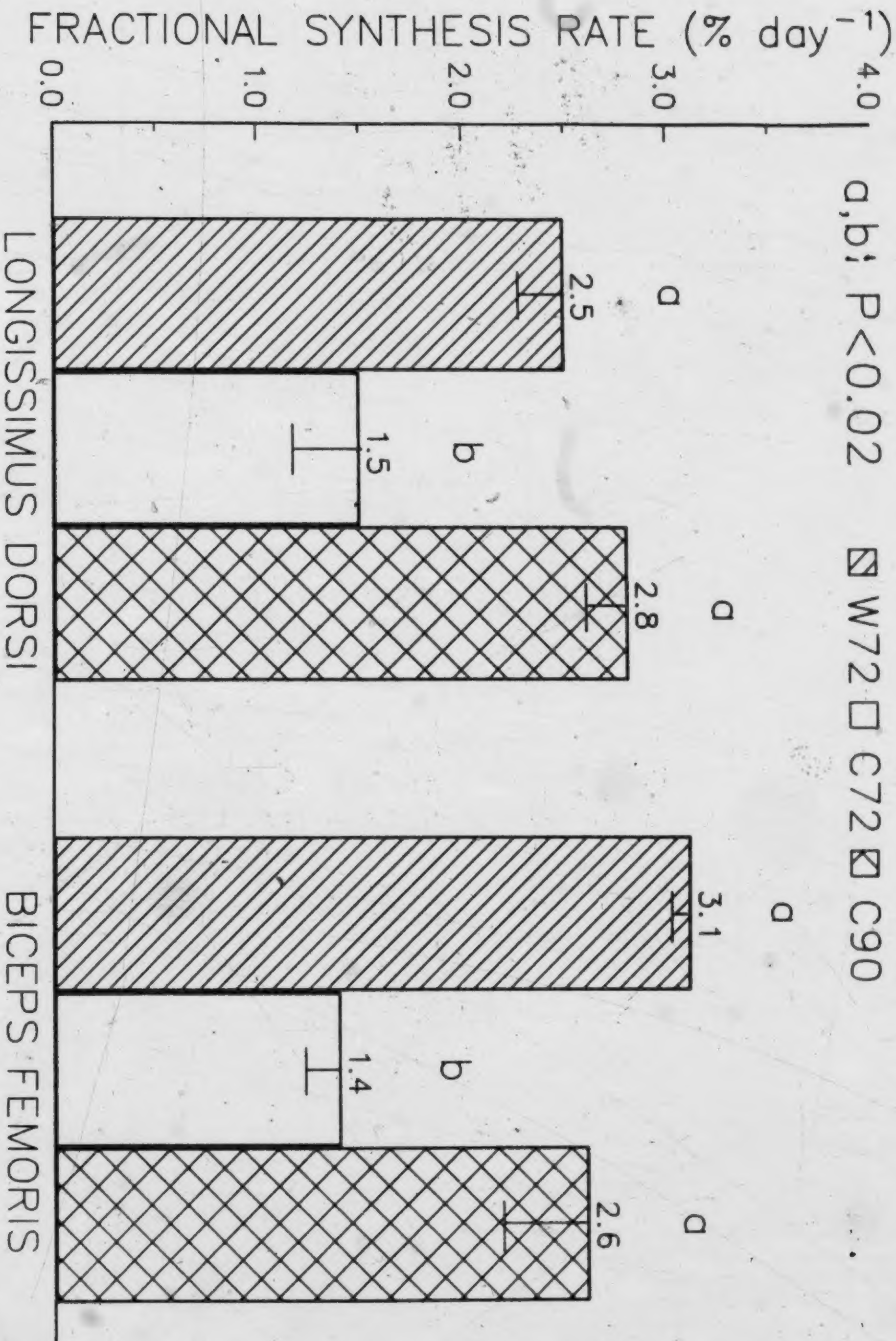

FRACTIONAL SYNTHESIS RATE (% day^{-1})
0.0
1.0
2.0
3.0
4.0
a,b: P<0.02
▨ W72 □ C72 ▩ C90
a
2.5
b
1.5
a
2.8
a
3.1
b
1.4
a
2.6
LONGISSIMUS DORSI
BICEPS FEMORIS

FIGURE II-2B: The fractional synthesis rate (% day^{-1}) of protein in kidney and skin from calves adapted to two environmental temperatures. Values are means of 3 to 4 animals in each group and bars indicate the SEM.

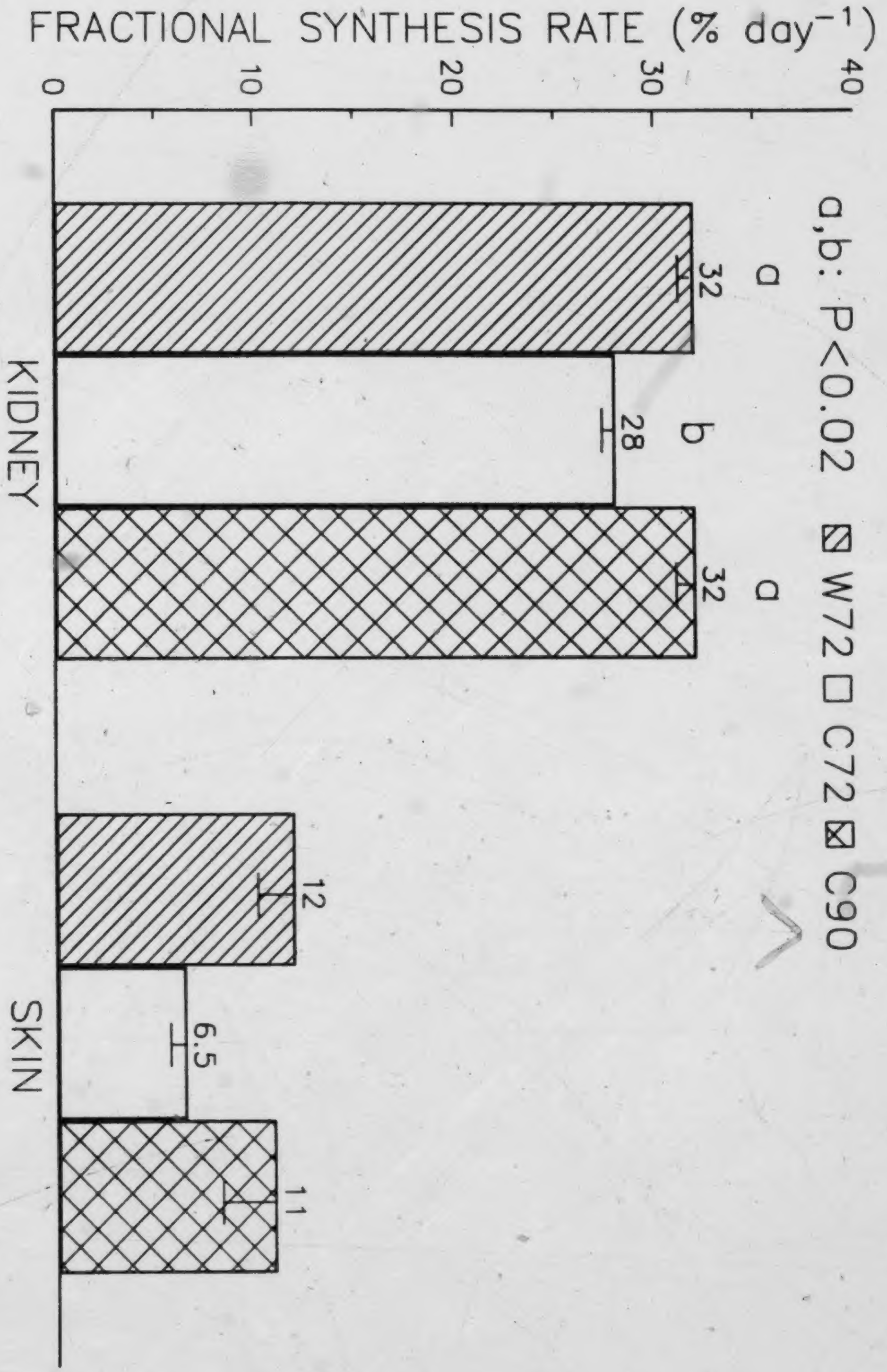

FRACTIONAL SYNTHESIS RATE (% day^{-1})
0
10
20
30
40
a,b: P<0.02 ▨ W72 ☐ C72 ☒ C90
a
32
b
28
a
32
KIDNEY
12
6.5
11
SKIN

FIGURE II-2C: The fractional synthesis rate (% day^{-1}) of protein in liver and heart from calves adapted to two environmental temperatures. Values are means of 3 to 4 animals in each group and bars indicate the SEM.

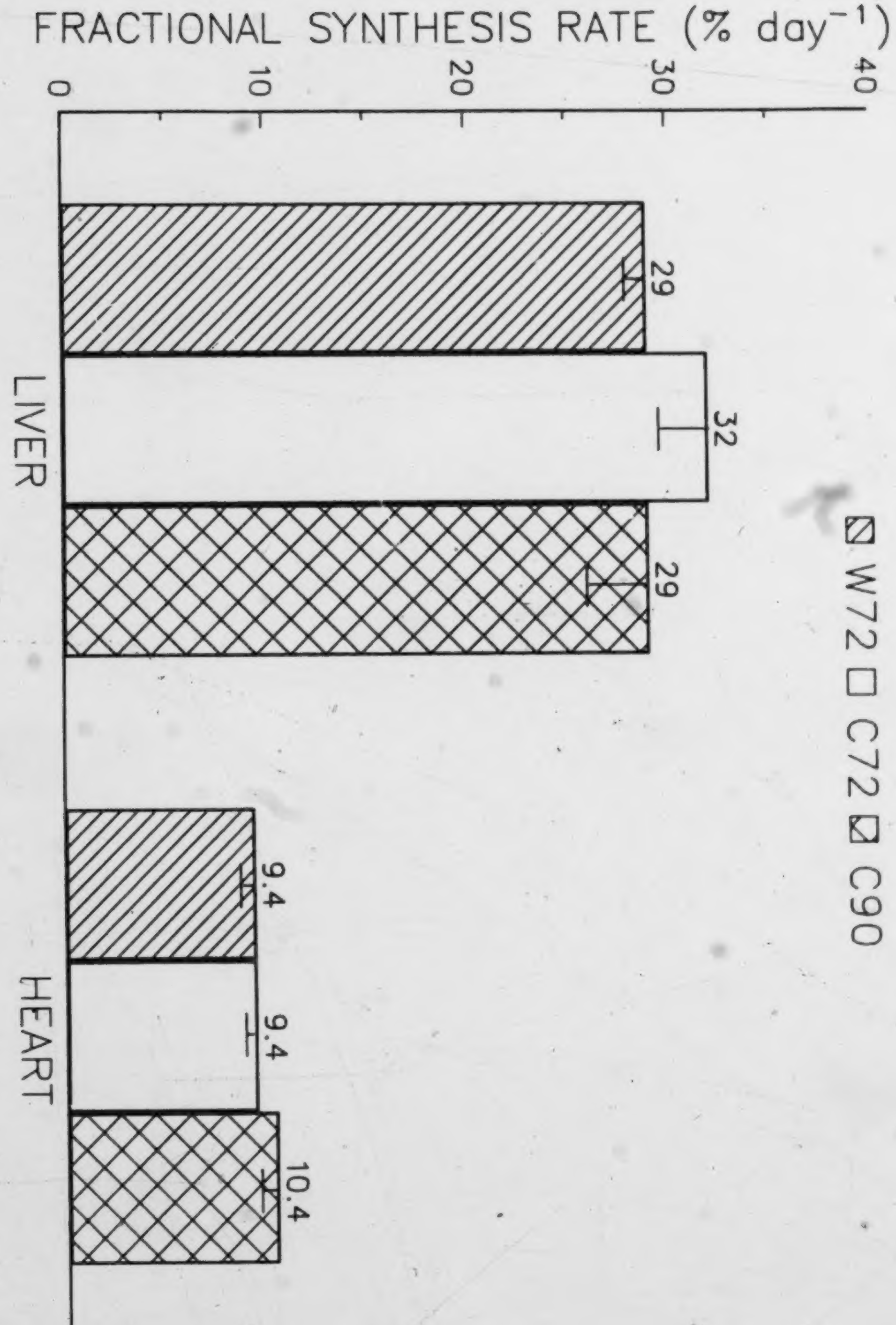

FRACTIONAL SYNTHESIS RATE (% day⁻¹)
0
10
20
30
40
LIVER
HEART
29
32
29
9.4
9.4
10.4
▨ W72 ☐ C72 ⊠ C90

FIGURE II-2D: The fractional synthesis rate (% day^{-1}) of protein in rumen wall and rumen papillae from calves adapted to two environmental temperatures. Values are means of 3 to 4 animals in each group and bars indicate the SEM.

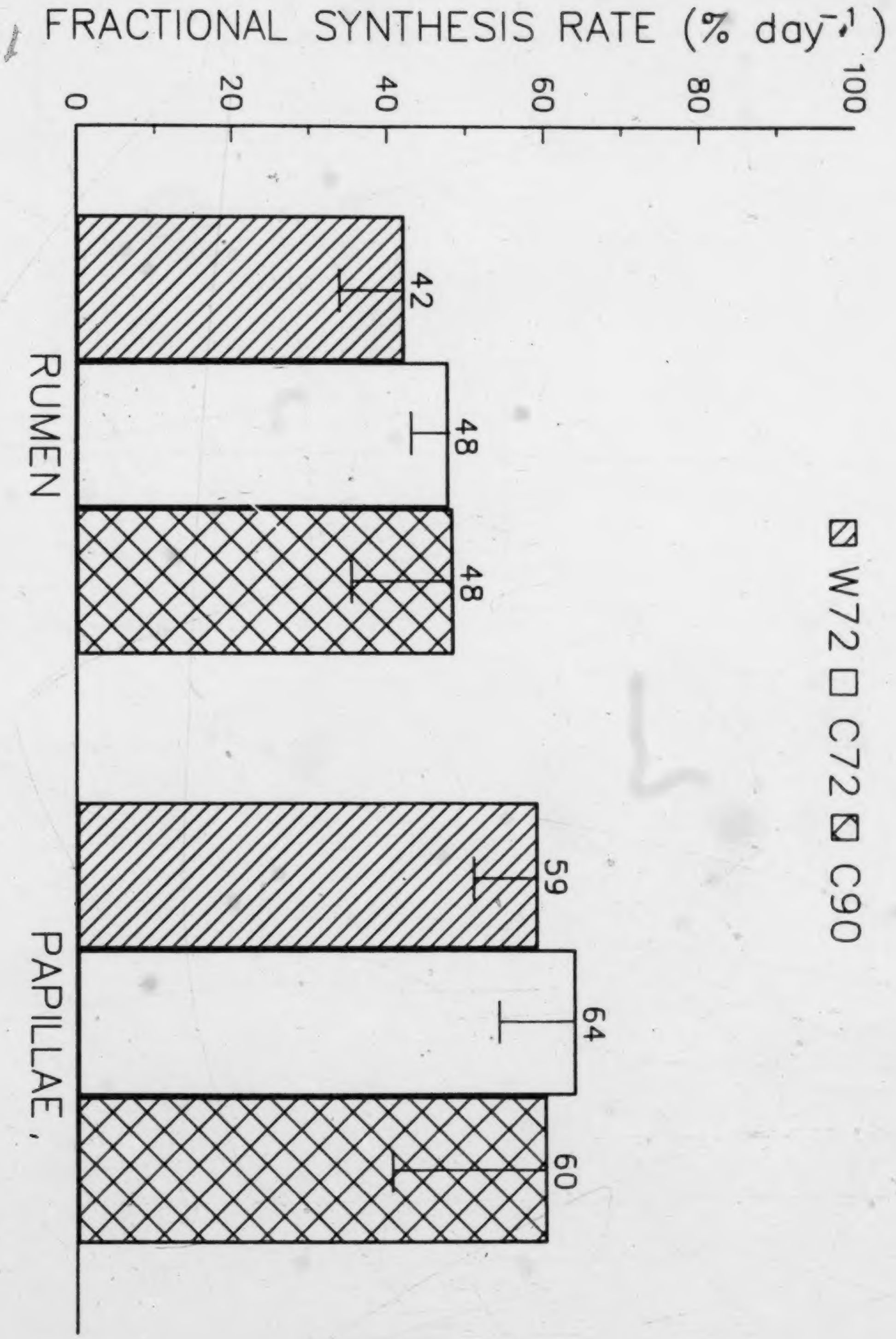

FRACTIONAL SYNTHESIS RATE (% day⁻¹)
0
20
40
60
80
100
▨ W72 ☐ C72 ▩ C90
42
48
48
59
64
60
RUMEN
PAPILLAE

FIGURE II-2E: The fractional synthesis rate (% day^{-1}) of protein in intestine and omasum from calves adapted to two environmental temperatures. Values are means of 3 to 4 animals in each group and bars indicate the SEM.

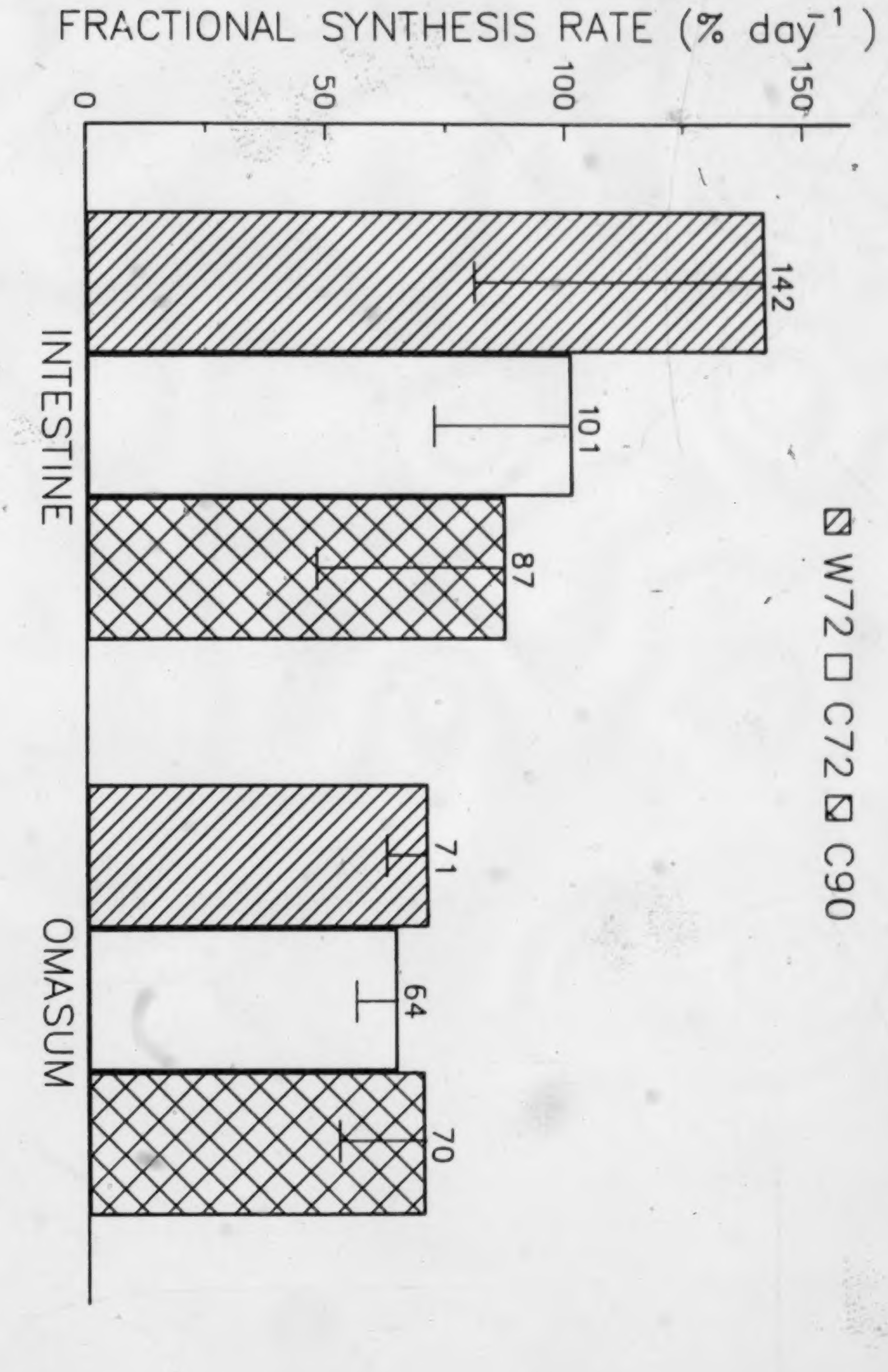

FRACTIONAL SYNTHESIS RATE (% day^{-1})
0
50
100
150
▨ W72 ☐ C72 ☒ C90
INTESTINE
OMASUM
142
101
87
71
64
70

FIGURE II-3: The relationship between the daily intake of nitrogen (g N day^{-1}) and absolute synthesis rates of protein in whole-body muscle or skin (g N day^{-1}). Points are individual values of calves in the C72 and C90 groups.

For muscle the equation of the line of best fit is:

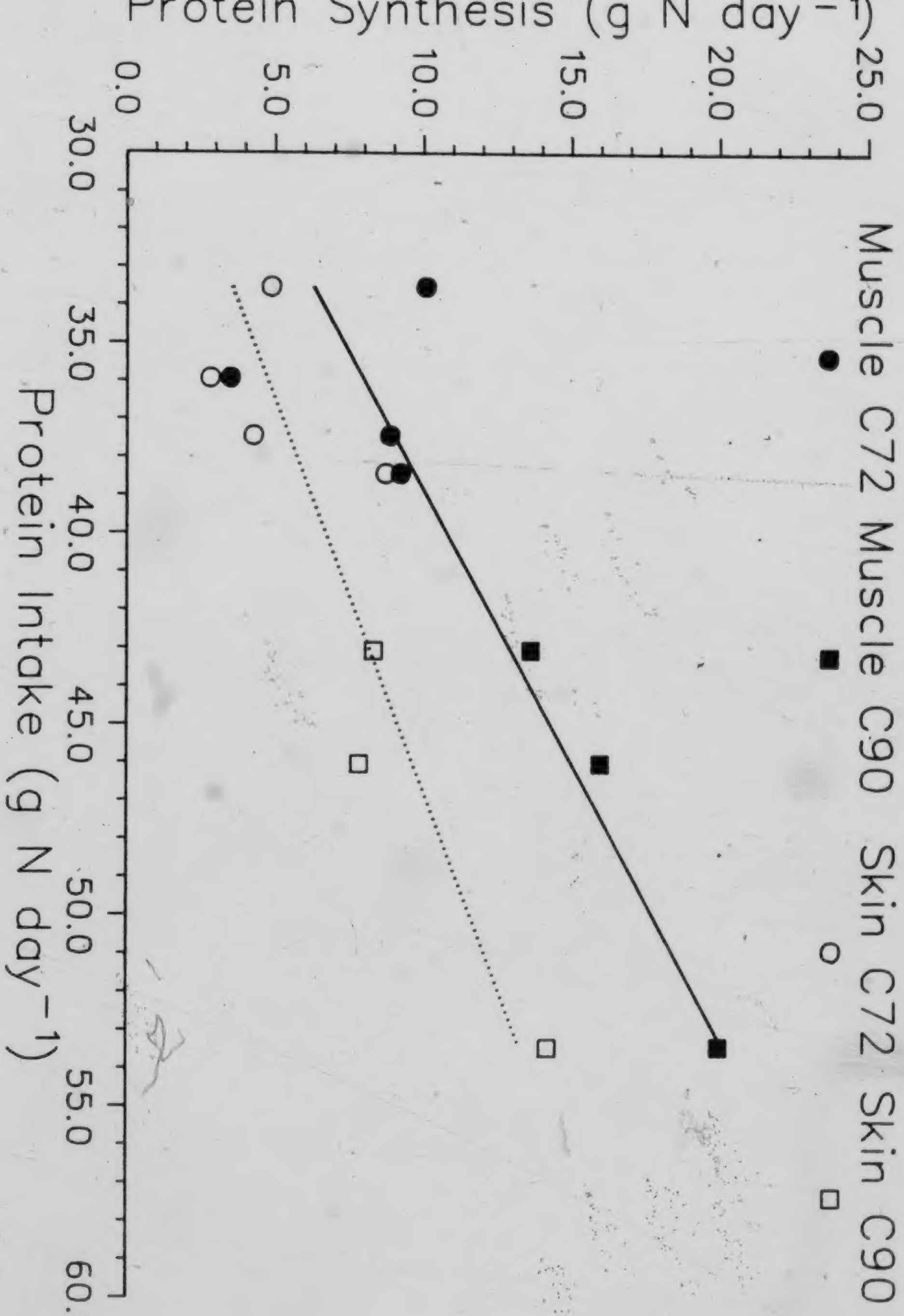
$$y = 0.687\ (\pm 0.158) - 16.8\ (\pm 6.58)\ x$$

where y is the absolute synthesis rate of protein and x is protein intake; $n = 7$, $P<0.01$, $r^2 = 0.79$.

For skin the equation of the line of best fit is:

$$y = 0.479\ (\pm 0.113) - 12.5\ (\pm 4.71)\ x$$

where y is the absolute synthesis rate of protein and x is protein intake; $n = 7$, $P<0.01$, $r^2 = 0.78$.


Protein Synthesis (g N day-1)
0.0
5.0
10.0
15.0
20.0
25.0
Protein Intake (g N day^{-1})
30.0
35.0
40.0
45.0
50.0
55.0
60.0
● Muscle C72
■ Muscle C90
○ Skin C72
□ Skin C90

LITERATURE CITED

Allison, J.B., and R.W. Wannemacher, Jr. 1965. The concept and significance of labile and over-all protein reserves of the body. Am. J. Clin. Nutr. 16: 445-452.

Ames, D.R., Brink, D.R., and C.L. Willms. 1980. Adjusting protein in feedlot diets during thermal stress. J. Anim. Sci. 50: 1-6.

Aoyagi, Y., Tasaki, I., Okumura, J.-I., and T. Muramatsu. 1988. Effect of low ambient temperature on protein turnover and heat production in chicks. Comp. Biochem. Physiol. 89A: 433-436.

Association of Organic and Agricultural Chemists. 1984. Official Methods of Analysis. 14th edition. Method 2.057. A.O.A.C., Arlington, USA.

Attaix, D., and M. Arnal. 1987. Protein synthesis and growth in the gastrointestinal tract of the young preruminant lamb. Br. J. Nutr. 58: 159-169.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Brown, J.G., Van Bueren, J., and D.J. Millward. 1984. Differential changes in free and total 3,5,3'-triiodothyronine in cold exposed rats. Proc. Nutr. Soc. 43: 74A.

Buttery, P.J., and E.F. Annison. 1973. Considerations of the efficiency of amino acid and protein metabolism in animals. In: The Biological Efficiency of Protein Production, J.G.W. Jones, ed., pp. 141-171. Cambridge University Press, Cambridge.

Buttery, P.J., and L. Bryan. 1986. Effect of diet and hormones on protein metabolism in muscle. In: Nuclear and Related Techniques in Animal Production and Health, pp. 457-468. IAEA, Vienna.

Dauncey, M.J., Ingram, D.L., James, P.S., and M.W. Smith. 1983. Modification by diet and environmental temperature of enterocyte function in piglet intestine. J. Physiol. 341: 441-452.

Davis, S.R., Barry, T.N., and G.A. Hughson. 1981. Protein synthesis in tissues of growing lambs. Br. J. Nutr. 46: 409-419.

Fuller, M.F. 1965. The effect of environmental temperature on the nitrogen metabolism and growth of the young pig. Br. J. Nutr. 19: 531-546.

Garlick, P.J., and G.E. Lobley. 1987. Dietary intake and protein turnover. Wiss. Z. WPU (Rostock) N.-Reihe. 36: 18-21.

Garlick, P.J., Millward, D.J., and W.P.T. James. 1973. The diurnal response of muscle and liver protein synthesis *in vivo* in meal-fed rats. Biochem. J. 136: 935-945.

Harris, C.I., and G. Milne. 1981. The urinary excretion of N^{τ}-methylhistidine by cattle: validation as an index of muscle protein breakdown. Br. J. Nutr. 45: 411-422.

Hartree, E.F. 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48: 422-427.

Jones, B.N., and J.P. Gilligan. 1983. *o*-Phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatog. 266: 471-482.

Lindsay, D.B., Dauncey, M.J., Barker, P.J., and D.L. Ingram. 1988. Protein synthesis and degradation after acclimation to different environmental temperatures and energy intakes. J. Therm. Biol. 13: 79-83.

Lobley, G.E., Milne, V., Lovie, J.M., Reeds, P.J., and K. Pennie. 1980. Whole body and tissue protein synthesis in cattle. Br. J. Nutr. 43: 491-502.

M^{c}Allister, T. 1987. Effect of temperature on protein turnover in the laboratory rat. M.Sc. Thesis. University of Alberta, Edmonton, Canada.

M^{c}Bride, B.W., Early, R.J., and R.O. Ball. 1989. Protein synthesis and the energy costs of Na$^+$, K$^+$-transport in tissues of somatotropin treated steers. In: Energy Metabolism of Farm Animals, Y. van der Honing and W.H. Close, eds., pp.107-110. EAAP publication No. 43. Pudoc, Wageningen.

M^{c}Lean, J.A. 1972. On the calculation of heat production from open-circuit calorimetric measurements. Br. J. Nutr. 27: 597-600.

Millward, D.J., Garlick, P.J., Nnanyelugo, D.O., and J.C. Waterlow. 1976. The relative importance of protein synthesis and breakdown in the regulation of muscle mass. Biochem. J. 156: 185-188.

Munro, H.N., and A. Fleck. 1969. Analysis for nitrogenous constituents. In: Mammalian Protein Metabolism, vol. III, p. 424-525. H.N. Munro, ed. Academic Press, New York.

Nakamura, H., and J.J. Pisano. 1976. Fluorescamine derivatives of histidine, histamine, and certain related imidazoles: unique fluorescence after heating in acid. Arch. Biochem. Biophys. 177: 334-335.

National Research Council. 1978. Nutrient Requirements of Dairy Cattle, 5th edition. National Academy of Sciences, Washington, D.C.

Newsholme, E.A. 1987. Substrate cycles and energy metabolism: Their biochemical, biological, physiological and pathological importance. In: Energy Metabolism of Farm Animals, P.W. Moe, H.F. Tyrrell, and P.J. Reynolds, eds., pp. 174-186. EAAP publication no. 32. Rowman and Littlefield, Totowa.

Nishizawa, N., Toyoda, Y., Noguchi, T., Hareyama, S., Itabashi, H., and R. Funabiki. 1979. N^{τ}-Methylhistidine content of organs and tissues of cattle and an attempt to estimate fractional catabolic and synthetic rates of myofibrillar proteins of skeletal muscle during growth by measuring urinary output of N^{τ}-methylhisitidine. Br. J. Nutr. 42: 247-252.

Preedy, V.R., M^{c}Nurlan, M.A., and P.J. Garlick. 1983. Protein synthesis in skin and bone of the young rat. Br. J. Nutr. 49: 517-523.

Rannels, D.E., Wartell, S.A., and C.A. Watkins. 1982. The measurement of protein synthesis in biological systems. Life Sci. 30: 1679-1690.

Read, S.M., and D.H. Northcote. 1981. Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Anal. Biochem. 116: 53-64.

Reeds, P.J., Cadenhead, A., Fuller, M.F., Lobley, G.E., and J.D. M^{c}Donald. 1980. Protein turnover in growing pigs. Effects of age and food intake. Br. J. Nutr. 43: 445-455.

Reeds, P.J., and M.F. Fuller. 1983. Nutrient intake and protein turnover. Proc. Nutr. Soc. 42: 463-471.

SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 edition. SAS Institute, Inc., Cary, USA.

Shipley, R.A., and R.E. Clark. 1972. Tracer Methods for In-Vivo Kinetics: Theory and Applications. Academic Press, New York.

SPSS Inc. 1988. SPSS-X User's Guide, 3$^{\underline{rd}}$ edition. SPSS Inc., Chicago, USA.

Steele, R.G.D., and J.H. Torrie. 1980. Principles and Prodedures of Statistics. M^{c}Graw-Hill Book Co., New York, USA.

Suzuki, O., and K. Yagi. 1976. A fluorimetric assay for β-phenylethylamine in rat brain. Anal. Biochem. 75: 192-200.

Thompson, J.R., Christopherson, R.J., and R.J. Early. 1987. Cold environmental temperatures increase the rate of skeletal muscle protein breakdown. 66th Annual Feeder's Day Report, pp. 52-54. Agriculture and Forestry Bulletin, University of Alberta, Edmonton, Canada.

Ugarte, J., Peron, N., and T.R. Preston. 1974. Rearing dairy calves by restricted suckling. V. Carcass composition and ruminal development of calves reared by restricted suckling. Cuban J.. Agric. Sci. 8: 211-218.

Wassner, S.J., Schlitzer, J.R., and J.B. Li. 1980. A rapid, sensitive method for the determination of 3-methylhistidine levels in urine and plasma using high-pressure liquid chromatography. Anal. Biochem. 104: 284-289.

Waterlow, J.C., Garlick, P.J., and D.J. Millward. 1978. Protein Turnover in Mammalian Tissues and in the Whole Body. Elsevier North Holland, Amsterdam.

Waterlow, J.C., and J.M.L. Stephen. 1968. The effect of low protein diets on the turnover rates of serum, liver and muscle proteins in the rat, measured by continuous infusion of L-[^{14}C]lysine. Clin. Sci. 35: 287-305.

Webster, A.J.F. 1976. Efficiencies of energy utilization during growth. In: Meat Animals: Growth and Productivity, D. Lister, D.N. Rhodes, V.R. Fowler, and M.F. Fuller, eds., pp. 89-102. Plenum Press, London.

Webster, A.J.F. 1981. The energetic efficiency of metabolism. Proc. Nutr. Soc. 40: 121-128.

Webster, A.J.F., Gordon, J.G., and R. M^{c}Gregor. 1978. The cold tolerance of beef and dairy type calves in the first weeks of life. Anim. Prod. 26: 85-92.

Young, B.A., Fenton, T.W., and J.A. M^{c}Lean. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 56: 1120-1125.

Young, B.A., Kerrigan, B., and R.J. Christopherson. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55: 17-22.

Yousef, M.K., and R.R.J. Chaffee. 1970. Studies on protein-turnover rates in cold-acclimated rats. Proc. Soc. Exptl. Biol. Med. 133: 801-804.

CHAPTER III

THE EFFECT OF A COLD ENVIRONMENT ON HORMONE CONCENTRATIONS AND KINETIC PARAMETERS IN HEIFERS AND CALVES

INTRODUCTION

In regions where ambient temperatures fall precipitously in the winter, animals increase heat production to maintain core body temperature. In order to meet the thermogenic demand, energy substrates are mobilized from body stores and *ad libitum* feed intake increases. These alterations in nutrient supply are likely to involve changes in endocrine parameters such as hormone concentrations or kinetics. In addition, hormonal alterations may also stimulate increased protein turnover, a thermogenic substrate cycle (Newsholme, 1987).

Using the euglycemic insulin clamp procedure, Weekes *et al.* (1983) showed that plasma glucose concentration and basal glucose flux were elevated in adult sheep exposed to 0°C, likely as a result of higher glucagon concentrations (Sasaki *et al.*, 1982). Several parameters of glucose metabolism such as rates of appearance and utilization were higher in the cold over a range of insulin infusion rates, suggesting that cold-exposure enhanced peripheral tissue responsiveness to insulin. In addition, insulin metabolic clearance rate was more rapid in the cold.

Cold-adaptation has been shown to affect other endocrine parameters in domestic animals. Along with its effects on protein accretion, growth hormone also plays a role in mobilizing energy substrates. However, few studies have examined effects of cold-adaptation on growth hormone concentrations or kinetics. In three cows adapted to -26°C for 3 weeks, growth hormone was increased in one animal during cold exposure, and in all animals for three weeks following a return to thermoneutral conditions (Olsen and Trenkle, 1973). Some effects of growth hormone may be mediated by insulin-like growth factor I (IGF-I), which can affect muscle growth as well as mimic the hypoglycemic actions of insulin (Zapf and Froesch, 1986). Plasma IGF-I concentrations were depressed in young pigs acclimated to 10°C compared with 35°C, especially those on a low level of feed intake (Dauncey *et al.*, 1990). Plasma thyroxine and triiodothyronine concentrations, which are usually elevated in cold-adapted animals, likely play a role in increasing heat production (Christopherson *et al.*, 1978).

Since the endocrine system constantly responds to changing conditions in order to maintain homeostasis, plasma concentrations of hormones are only static indicators of a dynamic system. Kinetic parameters, which delineate rates at which different aspects of hormone metabolism occur, are more accurate descriptors of the endocrine system.

Kinetic parameters may be calculated following a single hormone injection using principles outlined for radioactive tracers (Shipley and Clark, 1972). The analysis assumes that a hormone will be distributed into discrete compartments in the body which are maintained at a constant size by equal rates of input and output in a dynamic equilibrium known as steady state. The rate at which the hormone flows through the compartment at steady state is known as the irreversible loss or flux. Alterations in the size of the compartment or the inflow or outflow rates will have a bearing on the plasma concentrations of a hormone. Increased plasma concentrations of a hormone can actually decrease the number or binding affinity of its cell membrane receptors, affecting responsiveness of cellular substrate metabolism to the hormone (Young, 1980). In addition, the length of time a hormone remains in a compartment could affect its opportunities to interact with receptors on responsive tissues.

Since cold-adaptation was shown to affect plasma concentrations of various hormones, it was hypothesized that endocrine kinetic parameters may also be altered by cold-adaptation. In addition, since many hormones regulate protein turnover, endocrine alterations may also stimulate protein turnover in the cold thus contributing to increased heat production. The present experiment was designed to evaluate the effect of cold-adaptation on growth hormone and insulin kinetic parameters in heifers following a single injection, along with responses of other hormones and glucose to each hormone challenge.

MATERIALS AND METHODS

HEIFERS:

Animals and Feeding:

Ten Holstein heifers 1 year of age housed in groups of five in temperature-controlled environmental chambers initially were randomly assigned to either a warm (20.3 ± 0.4 °C) or cold (-17.2 ± 1.6 °C) environment during 2 periods of a crossover design. Heifers were allowed to adapt to temperatures for 7 weeks during which time they were fed barley grain at 17 g $kg^{-0.75}$BW (108 g crude protein kg^{-1}DM and 16.3 MJ GE kg^{-1}DM) each morning and alfalfa-brome hay was provided *ad libitum* (115 g crude protein kg^{-1}DM and 16.4 MJ gross energy kg^{-1}DM). Body weights were recorded prior to the week of blood sampling.

Heifers in this experiment previously participated in an experiment which examined the effect of environmental temperature on norgestomet (a synthetic progesterone) release from silastic ear implants. Heifers were not used in the present experiment until ear implants had been removed and one estrous cycle had been completed.

Experimental Design:

During each period of the crossover design, heifers received an injection of insulin or growth hormone on two consecutive days. A jugular catheter was inserted into each heifer 24 hours prior to the first sampling day and catheters were kept patent overnight with heparinized saline (200 Units mL^{-1}). During blood sampling, hay was removed and heifers were tethered. Blood samples (10 mL) were taken at -60, -45, -30, -15, -2, 1, 2, 3, 4, 6, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, and 180 minutes relative to the injection of bovine insulin (5 $\mu g\ kg^{-1}BW$; Sigma Chemical Co., St. Louis, USA) or a pituitary extract of bovine growth hormone (30 $\mu g\ kg^{-1}BW$; lot no. 035C, ICN Immunobiologicals, Cleveland, USA). Insulin or growth hormone was first dissolved in 2 mL of 0.01 N HCl or 20 mL of 0.025 M bicarbonate buffer, respectively, then made up to 100 mL with sterile saline. Blood was dispensed into test tubes containing 0.02 g Na_2EDTA and 150 μL of a 40 g L^{-1} solution of NaF, mixed, and kept on ice until it was centrifuged within 2 hours of sampling at 569 x *g* for 15 minutes at 4°C. Plasma was stored at -30°C until analyzed. Aliquots of plasma for glucagon radioimmunoassay contained the protease inhibitor Aprotinin (500 IU mL^{-1} plasma; Miles Laboratories, Mississauga, Canada).

A second injection of growth hormone was given 24 hours after the first. Serum samples were collected for analysis of insulin-like growth factor 1 (IGF-1) every 6 hours up to 72 hours following the first growth hormone injection.

Plasma glucose concentration was determined enzymatically using a modification of the glucose oxidase method developed by Trinder (1969). A Trinder reagent purchased from the Sigma Chemical Co. (St. Louis, USA) was reconsititued with distilled water then diluted 1:2 (v/v) with 0.005 M phosphate buffer (pH 7.0). A 3 mL aliquot of this dilute Trinder solution was added to 0.030 mL of plasma or standard, vortexed, and placed in a 37°C water bath for 20 minutes. After 5 minutes when samples had cooled to room temperature, absorbance at 490 nm was determined with a Brinkman PC800 dipping probe colorimeter. A standard curve was constructed using glucose standards prepared from a stock solution of

6.6667 g glucose dissolved in 100 mL of saturated benzoic acid.

CALVES:

Animals and feeding:

The calves which participated in the study of the effect of cold-adaptation on rates of tissue protein synthesis in Chapter II were also used in the present study. The care and feeding of the animals were as described in Chapter II and the plasma samples used in the present experiment were obtained during the continuous infusion of ^{3}H-phenylalanine, also as described in Chapter II.

HORMONE RADIOIMMUNOASSAYS:

Plasma samples were analyzed for insulin, IGF-I, growth hormone, glucagon, triiodothyronine, and thyroxine by radioimmunoassay. Insulin and growth hormone were iodinated according to the method outlined by De Boer and Kennelly (1989) except that 100 μL rather than 50 μL of potassium iodide solution were added to the growth hormone reaction mixture and 5 % (w/v) bovine serum albumin was used rather than a 2.5 % solution. IGF-I was iodinated by the same method as insulin.

Insulin:

Insulin was analyzed according to the method outlined by De Boer and Kennelly (1989). The gamma counting system used in this and all subsequent radioimmunoassays was a Cobra Model 5010 (Packard Instrument Co., Meriden, USA). The mean sensitivity of the assay was 0.137 ng mL^{-1}. Intraassay and interassay coefficients of variation for a sample averaging 2.50 ng mL^{-1} were 2.1 % and 24 %, respectively. Intraassay and interassay coefficients of variation for a sample averaging 13.4 ng mL^{-1}

were 7.6 % and 19 %, respectively.

Growth Hormone:

Growth hormone was analyzed according to the method outlined by De Boer and Kennelly (1989). The bovine somatotropin (USDA-bGH-B-1) used to construct the standard curves was a generous gift of Dr. Salvatore Raiti (National Hormone and Pituitary Program, National Institute of Diabetes, Digestive and Kidney Diseases, Baltimore, USA) and the antisera (anti-bGH2) was a generous gift of Dr. R. LaChance (Centre de Rechercher en Endocrinologie, Motechlaire, Canada). The mean sensitivity of the assay was 1.40 ng mL^{-1}. Intraassay and interassay coefficients of variation for a sample averaging 5.39 ng mL^{-1} were 4.18 % and 17.6 %, respectively. Intraassay and interassay coefficients of variation for a sample averaging 127 ng mL^{-1} were 4.33 % and 5.48 %, respectively.

Insulin-like Growth Factor I:

Insulin-like growth factor I was analyzed according to the method of Glimm *et al.* (1988). Following separation of IGF-I from its carrier protein using an acid-gel filtration, 10 μL of eluant were dispensed in triplicate into 12 x 75 mm disposable borosilicate glass tubes and dried completely in a vacuum centrifuge (Savant Speed-vac Concentrator Model SVC-200H; Savant Instruments Inc., Farmingdale, USA). Tubes for standards and non-specific binding were prepared in a similar manner with 10 μL of elution buffer (1.0 M acetic acid, BDH Chemicals LTD., Poole, U.K.; and 0.1 M NaCl, BDH Chemicals LTD., Poole, U.K.). Standard curves were constructed with synthetic insulin-like growth factor I (Lot No. 588C; Bachem Fine Chemicals, Inc., Torrance, USA). The antiserum (anti-somatomedin C, UBK 487) used in the radioimmunoassay was a generous gift of Dr. Salvatore Raiti (National Hormone and Pituitary Program, National Institute of Diabetes, Digestive and Kidney Diseases, Baltimore, USA). The mean sensitivity of the assay was 60 ng mL^{-1} for the heifers and 20 ng mL^{-1} for the calves. Intraassay and interassay coefficients of variation for a sample averaging 120 ng mL^{-1} were 2.2 % and 1.2 %, respectively. Intraassay and interassay coefficients of variation for a sample averaging 222 ng mL^{-1} were 2.8 % and 7.7 %, respectively.

Glucagon:

Glucagon was only assayed in samples collected after the insulin challenge. Glucagon was analyzed according to Harris *et al.* (1979) with some modifications. The antiserum (30 K anti-beef/pork glucagon; lot O4A, pool 1, lot 18) used in the radioimmunoassay was provided by Dr. Roger Unger (The University of Texas, Southwestern Medical School, Dallas, USA) and radioactive ^{125}I-glucagon was purchased (NEN Research Products, Boston, USA; Lot AJA1090). Volumes used in the assay system were: 100 μL sample or standard, 250 μL antiserum (1:115,000, containing 1-1.4 units of heparin and 400 KIU of trasylol), 250 μL of ^{125}I-glucagon (approximately 5000 c.p.m.). Separation of bound from free ^{125}I-glucagon was carried out by double antibody precipitation using 100 μL of goat antirabbit gamma globulin (1:25, Catalog No. 539844, Calbiochem, San Diego, USA) and 100 μL of normal rabbit serum (1:150). The mean sensitivity of the assay was 0.020 ng mL^{-1}. Intraassay and interassay coefficients of variation for a sample averaging 0.281 ng mL^{-1} were 3.6 % and 5.7 %, respectively.

Thyroid Hormones:

Total triiodothyronine and thyroxine were analyzed with a solid-phase [^{125}I] radioimmunoassay kit with antibody-coated tubes and human serum calibrators (Coat-a-Count Total T3 and T4; Diagnostic Products Corp., Los Angeles, USA).

STATISTICAL ANALYSIS:

Heifer hormone data:

Since it was assumed that insulin and growth hormone were distributed in two compartments, disappearance curves of hormone concentration as a fraction of administered dose were fit to an exponential equation comprising two components (Shipley and Clark, 1972) using a nonlinear iterative procedure (SAS, 1985). Kinetic parameters were calculated as outlined by De Boer *et al.* (1986). Distribution space (mL), which is the volume in which the hormone is distributed, was calculated from:

$$\text{Distribution space} = 1/(A_1 + A_2)$$

where A_1 and A_2 (mL^{-1}) are the zero time intercepts of the first and second components, respectively (see Figure III-1). Pool size (mg), or the total amount of hormone in the sampled compartment (i.e., plasma) was the product of the distribution space (mL) and the basal hormone concentration (ng mL^{-1}). Turnover rate ($minutes^{-1}$), or the proportion of hormone removed from the distribution space per unit time, was calculated from:

$$\text{Turnover rate} = H_1B_1 + H_2B_2$$

where $H_1 = A_1/(A_1 + A_2)$, $H_2 = A_2/(A_1 + A_2)$, and B_1 and B_2 are the rate constants for components 1 and 2, respectively. The half-life of each component was calculated as 0.693 divided by the respective rate constant. The metabolic clearance rate (mL hour^{-1}), or the volume of plasma cleared of hormone per unit time, was the product of distribution space (mL) and turnover rate (minutes^{-1}). The secretion rate into the plasma (μg hour^{-1}), or the amount of hormone secreted into the distribution space per unit time, was the product of distribution space (mL), turnover rate (minutes^{-1}), and basal hormone concentration (ng mL^{-1}).

Data were subjected to an analysis of variance using SAS (SAS, 1985). Means presented in tables are least-squares means and pooled standard error of the mean (SEM). For basal hormone concentrations, the model included temperature, period, and animal.

In the case of insulin challenge where data from both periods were used, the model for insulin kinetic parameters included temperature, period, and animal. For hormone and metabolite responses to insulin injection, the model included temperature, period, animal, and time and the interactions between temperature and time as well as period and time. Concentrations of hormones or metabolites in samples taken after the insulin challenge were compared to concentrations at time 0 using single degree of freedom comparisons (SAS, 1985).

In the case of the growth hormone challenge where only data from the second period were used due to a problem with the pH of the injection in the warm in the first period, the model for growth hormone kinetic parameters only included temperature as a main effect. For hormone and metabolite responses to growth hormone injection, the model included temperature, animal within temperature, time, and the interaction between temperature and time as main effects. In these analyses, the temperature was tested against animal within temperature. Concentrations of hormones or metabolites in samples taken after the growth hormone challenge were compared to concentrations at time 0 using single degree of freedom comparisons (SAS, 1985).

Calf homone data:

Data were subjected to an analysis of variance using SPSS-X (SPSS, 1988). Means were evaluated using the Student-Newman-Keuls multiple range test according to Steele and Torrie (1980).

Growth hormone parameters for each calf were evaluated according to the criteria outlined by Breier *et al.* (1986). Median plasma growth hormone concentration during the 8 hour infusion period was determined by SPSS-X (SPSS, 1988). A significant shift in growth hormone secretion occurred when the concentration of a given sample exceeded that of the previous sample by at least three times the interassay variation. A pulse was defined as a significant shift in secretion maintained for at least two consecutive samples above the median growth hormone concentration for each individual animal. In addition, there had to be more than two declining values between GH peaks. For each animal, the baseline growth hormone concentration during the 8 hour infusion period was calculated as the mean of all values falling below the median. In order to determine the area under growth hormone pulses, all values above the baseline were summed after subtraction of the baseline (SAS, 1985). The maximum growth hormone value attained in a peak was defined as the peak height.

RESULTS

HEIFERS:

Basal Concentrations of Hormones and Metabolites:

Basal plasma concentrations of all hormones (Table III-1) except growth hormone and IGF-I were significantly higher in the cold environment ($P<0.05$). Plasma glucose concentration was not significantly affected by temperature.

Insulin Kinetic Parameters:

Following the injection, insulin concentration increased to approximately 200 ng mL^{-1} and declined exponentially, returning to baseline within sixty minutes. Environmental temperature did not significantly influence any of the insulin kinetic parameters (Table III-2). Body weight was significantly lower in the cold environment ($P<0.05$).

Responses of Hormones and Metabolites to Insulin Challenge:

Within 10 minutes of the insulin injection, plasma glucose concentration dropped sharply (Figure III-2), and remained significantly lower than the time 0 value for at least 120 minutes post-injection ($P<0.05$). Overall, plasma glucose concentration was higher in the cold than in the warm ($P<0.001$) and there was a trend for the interaction between temperature and time to be significant ($P=0.07$), indicating a possible effect of environment on the time course of glucose response to insulin injection.

Plasma glucagon concentration peaked at approximately 50 minutes after insulin injection (Figure III-3), returning to baseline after an additional 55 minutes. Overall, the concentration of glucagon was higher in the cold ($P<0.001$), but there was no interaction between temperature and time.

Plasma growth hormone concentration did not respond to insulin until later in the post-injection period (Figure III-4), at which time it was elevated for at least 90 minutes. There was neither an effect of temperature nor any interaction between temperature and time. No growth hormone peaks were observed in the heifers.

Growth Hormone Kinetic Parameters:

Only data from period 2 of the crossover design were used to determine growth hormone kinetic parameters and hormone and metabolite responses to growth hormone injection.

Growth hormone kinetic parameters are shown in Table III-3. The zero time intercept of the slow component and its respective time constant were significantly lower in the cold environment ($P<0.05$), and a similar trend was seen for the time constant of the fast component ($P<0.10$). All other kinetic parameters were unaffected by environmental temperature.

Responses of Hormones and Metabolites to Growth Hormone Challenge:

Approximately 25 minutes post-injection, plasma glucose decreased (Figure III-5), but then rebounded to higher-than-baseline values by 180 minutes post-injection. As with the insulin challenge, overall plasma glucose was higher in the cold than in the warm environment ($P<0.05$), but there was no interaction between temperature and time.

Within 10 minutes of the growth hormone injection plasma insulin concentration rose (Figure III-6), but then dropped to lower-than-baseline values after an additional 20 minutes. There was neither an effect of temperature nor any interaction between temperature and time.

Although serum IGF-I concentration did rise slightly after the first growth hormone injection (Figure III-7), the increase was not significantly different from time 0 until after the second injection 24 hours later. In the warm, IGF-I fell to baseline levels 24 hours after the second injection, but in the cold there was an additional rise in IGF-I which started 36 hours after the second injection (60 hours after the first injection). There was a trend ($P<0.10$) for both temperature and the interaction between temperature and time to significantly affect the serum IGF-1 response to growth hormone injection.

CALVES:

Growth Hormone Parameters:

Plasma growth hormone concentration fluctuated in calves in a pulsatile manner (Figure III-8). The apparent pulsatile release can be quantitated by measuring the number, height, and area under growth hormone peaks (Table III-4). Environmental temperature did not influence any of the growth hormone parameters measured.

Basal Hormone Levels:

Basal levels of insulin, IGF-I, tiiodothyronine, and thyroxine (Table III-5) were unaffected by treatment in the calves.

DISCUSSION

HEIFERS:

Methodology Used to Determine Kinetic Parameters:

Kinetic parameters of insulin and growth hormone were calculated using principles outlined for radioactive tracers using a two-compartment model by Shipley and Clark (1972). In the present experiment, the plasma pool was the sampled compartment, but the second pool is not well defined. A steady state was assumed during the sampling period following the hormone injection. This is likely a valid assumption when a small amount of radioactive tracer is administered. However, the quantity of injected hormone substantially elevated its plasma concentration, and it is unknown what effect this perturbation may have had on calculated kinetic parameters.

A further assumption necessary for the analysis is that outflow of a hormone from a compartment following a single injection can be described by exponential equations. The fraction of hormone in a compartment or pool that is lost per unit time is known as the fractional rate constant (Shipley and Clark, 1972). On a semilog plot of time on the x axis versus the natural log of the hormone concentration as a fraction of the injected dose on the y axis, the slope of the line for a one compartment model will equal k, the fractional rate constant, and the zero time intercept will equal the hormone concentration (mg mL^{-1}) as a fraction of the injected dose (mg) at time zero. If more than one pool is involved, the curve will be a composite of the individual component curves (see Figure III-1). From the fractional rate constants and the zero time intercepts, the remainder of the kinetic parameters can be calculated.

Due to the exponential nature of the curve, data points obtained in the first few minutes of sampling have an enormous influence on the predicted zero-time intercepts and rate constants. Thus, any slight inaccuracies in sampling time will amplify variability in those parameters as well as values subsequently calculated from them.

Basal Plasma Hormone and Metabolite Concentrations:

Elevated plasma insulin and glucagon concentrations (Table III-1) after adaptation to a cold environment have been reported for Holstein calves exposed to 2°C for a week (Takahashi *et al.*, 1984) . However, in adult sheep acclimated to 0°C from 4-19 days, basal plasma insulin and glucagon levels were unchanged (Sasaki *et al.*, 1982), and in rats plasma insulin levels were reduced for the duration of cold-exposure (Vallerand *et al.*, 1983). Possibly, elevated plasma catecholamine concentrations could have played a role in the effect of cold on insulin and glucagon. Increased ß-adrenergic activity could stimulate insulin and glucagon secretion in the cold, and increased α-adrenergic activity may also elevate glucagon (Sasaki *et al.*, 1982). Elevated glucagon concentrations could also act synergistically with catecholamines to increase gluconeogenesis in a cold environment (Brockman and Laarveld, 1986).

In the present study, basal plasma glucose concentration was unchanged in the cold environment (Table III-1), possibly because elevated insulin concentrations were counteracted by greater glucagon concentrations. Plasma glucose is usually increased in the cold (Takahashi *et al.*, 1984; Sasaki *et al.*, 1982; Weekes *et al.*, 1983).

probably due to enhanced gluconeogenesis (Weekes *et al.*, 1983) required to supply glucose as an energy substrate in the cold (M^c^Kay, 1974). However, basal glucose concentration was unchanged in ewes conditioned to -2°C for 6 weeks, even though there were significant increases in glucose flux and utilization in the cold compared with 18°C (M^c^Kay, 1974).

Basal concentrations of growth hormone were not influenced by cold-adaptation (Table III-1). With a limited number of animals, Olsen and Trenkle (1973) reported that growth hormone was not increased by cold exposure for 3 weeks at -26°C in two out of three cows, although growth hormone did increase in all animals when they returned to 23°C. This rebound in growth hormone concentration following cold-exposure is supported by a similar finding in humans, where growth hormone concentration was not changed during a short (1-2 hour) cold exposure to 4°C, but rose rapidly following rewarming to 23°C (Okada *et al.*, 1970). The mechanism by which rewarming increases growth hormone levels is unknown. Underfed pregnant ewes housed in an open barn and exposed to -2 to -10°C exhibited increased growth hormone after shearing when it is likely they were cold-stressed, although nutritional stress may have been a greater stimulus for growth hormone secretion (Blom *et al.*, 1976).

Plasma thyroid hormones, which are thought to play a role in increasing heat production in response to cold stress, were also enhanced by exposure to 2-5°C for 28 days in shorn wethers (Christopherson *et al.*, 1978). Insulin in the cold may have been influenced by thyroxine, as thyroxine treatment in adult sheep has been shown to elevate plasma insulin (Godden and Weekes (1984).

Insulin Challenge:

Insulin kinetic parameters (Table III-2) for heifers were similar in many respects to those reported for lactating cows (De Boer and Kennelly, 1989) except that values of A_1 and A_2 were higher, and the half-life of the slow component was shorter. Weekes *et al*. (1983) found a higher insulin metabolic clearance rate in sheep exposed to a cold environment of 0°C for 7-23 days compared to those exposed to 20°C. Inhibition of insulin secretion in the cold (Sasaki *et al*., 1982) and increased metabolic clearance rates may have contributed to lower basal plasma insulin levels reported by Weekes *et al*. (1983).

The effect of insulin injection on glucose concentration (Figure III-2) in the present experiment was similar to the response reported for adult sheep exposed to 0°C for 4-19 days (Sasaki *et al*., 1982). In that experiment, both the initial drop in glucose concentration from basal values and glucose concentration *per se* during the response period were greater in cold-exposed animals. A possible effect of cold-adaptation on the time course of glucose response to insulin injection is also indicated in the present experiment by the trend toward a significant interaction between temperature and time. Although basal plasma glucose concentrations were not affected by temperature in the present experiment, glucose concentration remained higher and returned to baseline earlier in the post-injection period in the cold-adapted heifers. Glucose production likely exceeded glucose utilization by a larger margin in the cold compared with the warm and both rates were probably elevated in the cold (Weekes *et al*., 1983).

Peak glucagon response (Figure III-3) to insulin-induced hypoglycemia was higher than previously reported for sheep adapted to 0°C for 4-19 days (Sasaki *et al.*, 1982). This could indicate a higher responsiveness in pancreatic glucagon secretion in the heifers in the present experiment, especially those in the cold. More rapid recovery of plasma glucose concentration following the insulin injection could also indicate a greater responsiveness of hepatic gluconeogenesis to glucagon, which would involve alterations in post-receptor events in the liver (Weekes *et al.*, 1983).

In sheep, insulin-induced hypoglycemia was a potent stimulus for growth hormone secretion, with maximum levels exceeding 25 ng mL^{-1} (Machlin *et al.*, 1967). Trenkle (1971) also reported that in 12 hour-fasted sheep, insulin-induced hypoglycemia resulted in a doubling of plasma growth hormone concentrations from 5 ng mL^{-1} to about 12 ng mL^{-1}. In the present experiment, growth hormone response to insulin injection was not of the same magnitude (Figure III-4), possibly because the heifers were fed or because the hypoglycemia was not as severe. As with basal growth hormone concentrations, there was no influence of temperature on the growth hormone response to insulin injection. Lack of change in plasma concentrations of growth hormone does not preclude alterations in tissue responsiveness or sensitivity to this hormone. For example, increased responsiveness to growth hormone in the cold could contribute to mobilization of energy substrates (Brockman and Laarveld, 1986).

Growth Hormone Challenge:

The growth hormone injection for the group of heifers in the warm in Period 1 was made up in acidic rather than alkaline buffer, and did not dissolve well. Even when growth hormone kinetic parameters were adjusted to the actual concentration of hormone injected, values were still an order of magnitude different from those for all other animals, regardless of treatment. Thus, it was obvious that those values were affected by the injection itself, possibly due to denaturation of growth hormone by the alkaline pH. It was considered that this invalidated any attempt at treatment comparisons for Period 1. Therefore, only data from Period 2 were used.

Growth hormone kinetic parameters (Table III-3) are similar to those reported for dairy cows on a high protein diet (De Boer and Kennelly, 1989), although values for A_1 and A_2 were higher in the present experiment. As a result, the calculated distribution space was smaller in the heifers, which in turn lead to a smaller calculated pool size. Cold-adaptation reduced A_2 and B_2, which are the zero time intercept and the rate constant of the slow component, respectively. The distribution space, determined as $1/(A_1 + A_2)$, was not altered by environmental temperature. Although the proportion of $(A_1 + A_2)$ contributed by A_1 appeared to increase in the cold and the proportion due to A_2 actually did decrease, the sum of the two zero time intercepts, $(A_1 + A_2)$ was similar in both environments. This suggests that growth hormone may be distributed differently at time zero between the two compartments in warm- and cold-adapted heifers possibly due to a the fact that both rate constants were slower in the cold. Even though the rate constants were slower in the cold, there were no significant differences in the half-life of either component.

Declining plasma glucose concentrations during the first hour following a single injection of growth hormone have been reported for 24 hour-fasted sheep by Bassett and Wallace (1966). Likely, elevated insulin concentrations at ten minutes post-injection (Figure III-6) resulted in the transient decreases in plasma glucose in response to growth hormone in the present experiment (Figure III-5). After the initial rise in insulin concentration, values fell to baseline levels and then dropped below baseline, probably in response to declining plasma glucose concentrations. Plasma insulin and glucose concentrations both recovered to baseline values by 90 minutes post-injection. However, near the end of the sampling period, both hyperinsulinemia and hyperglycemia were observed, probably due to insulin resistance and decreased peripheral glucose utilization (Kipnis *et al.*, 1969). These delayed effects, also known as the diabetogenic effects of growth hormone, may actually be properties of other pituitary extracts present in the growth hormone preparation, as highly purified preparations do not acutely affect insulin and glucose concentrations (Bauman and M^{c}Cutcheon, 1985). The effects of cold-adaptation on the responses of glucose and insulin to growth hormone injection are opposite to its effects on their basal levels, i.e., overall glucose concentration was higher in the cold and insulin was not affected by temperature.

Elsasser *et al*. (1989) observed a similar pattern in IGF-I in response to a single growth hormone injection as the one seen in Figure III-7, with the peak in IGF-I occurring about 12-24 hours after the injection with plasma concentrations increasing by about 30 %. In the present experiment, in addition to the trend for overall IGF-I concentrations to be higher in the cold, there was also a trend for the response to growth hormone to follow a different pattern in the cold. This was especially evident between 60 and 72 hours after the first growth hormone injection, when IGF-I rose above baseline again in cold even in the absence of a growth hormone injection. The latter did not occur in the warm. Increased IGF-I concentrations in the cold may exert insulin-like effects on glucose utilization and may help maintain growth in the cold (Zapf and Froesch, 1986). However, elevated basal and growth hormone-induced concentrations of IGF-I in the cold are not supported by findings that IGF-I was depressed in pigs acclimated to 10°C for 10 weeks compared with 35°C (Dauncey *et al.*, 1990). This may indicate a species difference or a difference in the degree of cold stress experienced by the pigs versus the heifers.

CALVES:

Growth Hormone Parameters:

Growth hormone concentrations were higher in the calves than in the heifers. Unlike the case for the heifers, plasma growth hormone levels were pulsatile in nature (see Figure III-8). Baseline growth hormone concentrations and peak height were higher, and number of peaks and area under the growth hormone pulses were lower than respective values reported for 10-month-old Angus steers (Breier *et al.*, 1986). Most likely these differences are due to the age of the animals involved. The lower peak area is probably a function of a reduced number of peaks occurring during the sampling period. Peak number may be lower due to differences in the sampling frequency since Evans *et al.* (1987) reported in man more peaks are detected with more frequent sampling.

Other Hormonal Parameters:

Nutritional factors have been shown to be important in regulating IGF-I levels in growing steers (Breier *et al.*, 1986; Elsasser *et al.*, 1989). As feed becomes more restricted, the regulatory relationship between growth hormone and IGF-I is uncoupled, such that IGF-I concentrations are low at a time when growth hormone is high, indicating a growth hormone resistance (Breier *et al.*, 1986). This growth hormone resistance is most likely an attempt to mobilize energy stores via the lipolytic effect of growth hormone. In the above references, the most striking differences were seen when steers were in a negative nitrogen and/or energy balance. Even though calves in the C72 group still gained weight, they could be considered nutritionally stressed, and would therefore be expected to show higher growth hormone levels and lower IGF-I levels. Numerically, growth hormone appeared to be elevated and IGF-I appeared to be depressed in the C72 group (see Tables III-4 and III-5), but these trends were not significant, likely due to the large variability between animals. In a similar experiment with pigs, a group at 10°C on a high level of intake had similar IGF-I levels as a group at 35°C on a low level of intake, while a group at 10°C on a low level of intake had much lower IGF-I plasma concentrations of IGF-I than either of the other two groups (Dauncey *et al.*, 1990). It has been postulated that changes in insulin and thyroid hormones could mediate the effects of nutrition on growth hormone/IGF-I (Dauncey *et al.*, 1990; Elsasser *et al.*, 1989); however, treatment had no significant effect on plasma levels of these hormones in the calves in the present study.

CONCLUSION:

It appears that hormonal adaptations in the cold are directed toward increasing glucose production and use, particularly increased concentrations of insulin, IGF-I, and glucagon. Although insulin kinetic parameters were not influenced by temperature, the zero-time intercept and the rate constant of the slow growth hormone component were significantly depressed in cold-adapted heifers. Further experiments are required to determine if these effects of cold-adaptation on hormone concentrations and kinetic parameters are correlated with changes in tissue responsiveness or sensitivity. In the calves, no hormonal changes were observed as a result of cold-adaptation, although results suggested an inverse relationship between growth hormone and IGF-I in the C72 group. More information is required to clarify the effects of temperature and nutrition on growth hormone secretion and IGF-I production in growing ruminants. In addition, altered responsiveness or sensitivity to insulin, IGF-I, or growth hormone may mediate the depression in muscle protein synthesis observed in cold-adapted calves on the low level of intake.

TABLE III-1: Basal (pre-injection) concentrations of insulin, glucagon, growth hormone, thyroxine (T_4), triiodothyronine (T_3), and glucose in heifers adapted to warm and cold environments.

HORMONE	WARM	COLD	SEM
n	10	10	
Insulin (ng mL^{-1})	1.87^a	2.16^b	0.06
Glucagon (pg mL^{-1})	218^a	259^b	11
Growth Hormone (ng mL^{-1})	5.41	5.63	0.462
Triiodothyronine (ng mL^{-1})	1.75^a	2.25^b	0.04
Thyroxine (ng mL^{-1})	61.9^a	68.0^b	1.3
Glucose (mg mL^{-1}) (before insulin)	0.883	0.897	0.011
Glucose (mg mL^{-1}) (before growth hormone)	0.868	0.934	0.022

^{a,b}: Means in the same row with different superscripts are significantly different ($P<0.05$).

TABLE III-2: Insulin kinetic parameters in heifers adapted to warm and cold environments.

PARAMETER	WARM	COLD	SEM
n	10	10	
A_1, $mL^{-1} \times 10^4$	0.562	0.576	0.090
A_2, $mL^{-1} \times 10^4$	0.319	0.269	0.034
B_1, $minutes^{-1}$	0.462	0.431	0.102
B_2, $minutes^{-1}$	0.086	0.102	0.029
Turnover rate, $minutes^{-1}$	0.309	0.281	0.063
Half life, minutes			
Fast	3.41	2.35	0.49
Slow	9.30	10.11	1.40
Distribution Space, $L \times (100\ kg)^{-1}$	3.20	2.95	0.28
Metabolic Clearance Rate, $L \times (hour \times 100\ kg)^{-1}$	43.7	50.0	7.9
Secretion Rate, $\mu g \times (hour \times 100\ kg)^{-1}$	83.1	100.9	14.6
Pool Size, μg	25.6	27.3	2.5
Basal Insulin, ng mL^{-1}	1.87^a	2.16^b	0.06
Body Weight, kg	430^a	424^b	2

A_1 and A_2 are the zero time intercepts of the fast and slow components, respectively.

B_1 and B_2 are the rate constants of the fast and slow components, respectively.

^{a,b}: Means in the same row with different superscripts are significantly different ($P<0.05$).

TABLE III-3: Growth hormone kinetic parameters in heifers adapted to warm and cold environments. Means are least squares means. (Only data from Period 2 shown).

PARAMETER	WARM	COLD	SEM
n	5	5	
A_1, $mL^{-1} \times 10^4$	0.400	0.638	0.116
A_2, $mL^{-1} \times 10^4$	0.460^a	0.130^b	0.088
B_1, $minutes^{-1}$	0.200^c	0.089^d	0.039
B_2, $minutes^{-1}$	0.031^a	0.020^b	0.003
Turnover rate, $minutes^{-1}$	0.118	0.077	0.022
Half life, minutes			
Fast	6.33	8.20	2.13
Slow	24.0	40.8	7.3
Distribution Space, L x $(100\ kg)^{-1}$	2.71	2.97	0.19
Metabolic Clearance Rate, L x $(hour \times 100\ kg)^{-1}$	17.7	13.4	2.5
Secretion Rate, µg x $(h \times 100\ kg)^{-1}$	79.2	59.0	10.3
Pool Size, µg	58.3	61.5	11.2
Basal Growth Hormone, ng mL^{-1}	4.90	4.50	0.76
Body Weight, kg	441	463	32

A_1 and A_2 are the zero time intercepts of the fast and slow components, respectively.

B_1 and B_2 are the rate constants of the fast and slow components, respectively.

[a,b]: Means in the same row with different superscripts are significantly different ($P<0.05$).

[c,d]: Means in the same row with different superscripts tend to be different ($P<0.10$).

TABLE III-4: Growth hormone (GH) parameters of calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
Basal GH Concentration (ng mL^{-1})	9.03 ± 2.51	14.42 ± 4.17	6.78 ± 0.54	0.27
Number of Peaks in 8 hours	2.25 ± 0.48	2.25 ± 0.63	3.00 ± 1.15	0.74
Average Peak Height (ng mL^{-1})	68.18 ± 30.79	38.49 ± 8.69	31.65 ± 3.46	0.45
Area Under GH Peaks (ng x minutes) x mL^{-1}	74.2 ± 14.2	43.3 ± 7.2	51.2 ± 13.2	0.20

TABLE III-5: Basal Thyroxine (T_4), Triiodothyronine (T_3), and insulin-like growth factor 1 (IGF-I) concentrations of calves adapted to two environmental temperatures (mean ± SEM).

	W72	C72	C90	P VALUE
T_3 (ng mL^{-1})	0.973 ± 0.083	1.093 ± 0.082	1.263 ± 0.084	0.12
T_4 (ng mL^{-1})	55.08 ± 5.01	57.81 ± 7.22	70.63 ± 5.41	0.25
IGF-I (ng mL^{-1})	123.18 ± 28.73	80.12 ± 28.73	129.97 ± 33.18	0.47

FIGURE III-1: The insulin disappearance curve for heifer 603 in the cold environment in Period 2 after a single intravenous injection. The composite curve is the sum of the curves of the fast and slow components. A_1 and A_2 are the zero time intercepts of the fast and slow components, respectively, and B_1 and B_2 are their respective rate constants.

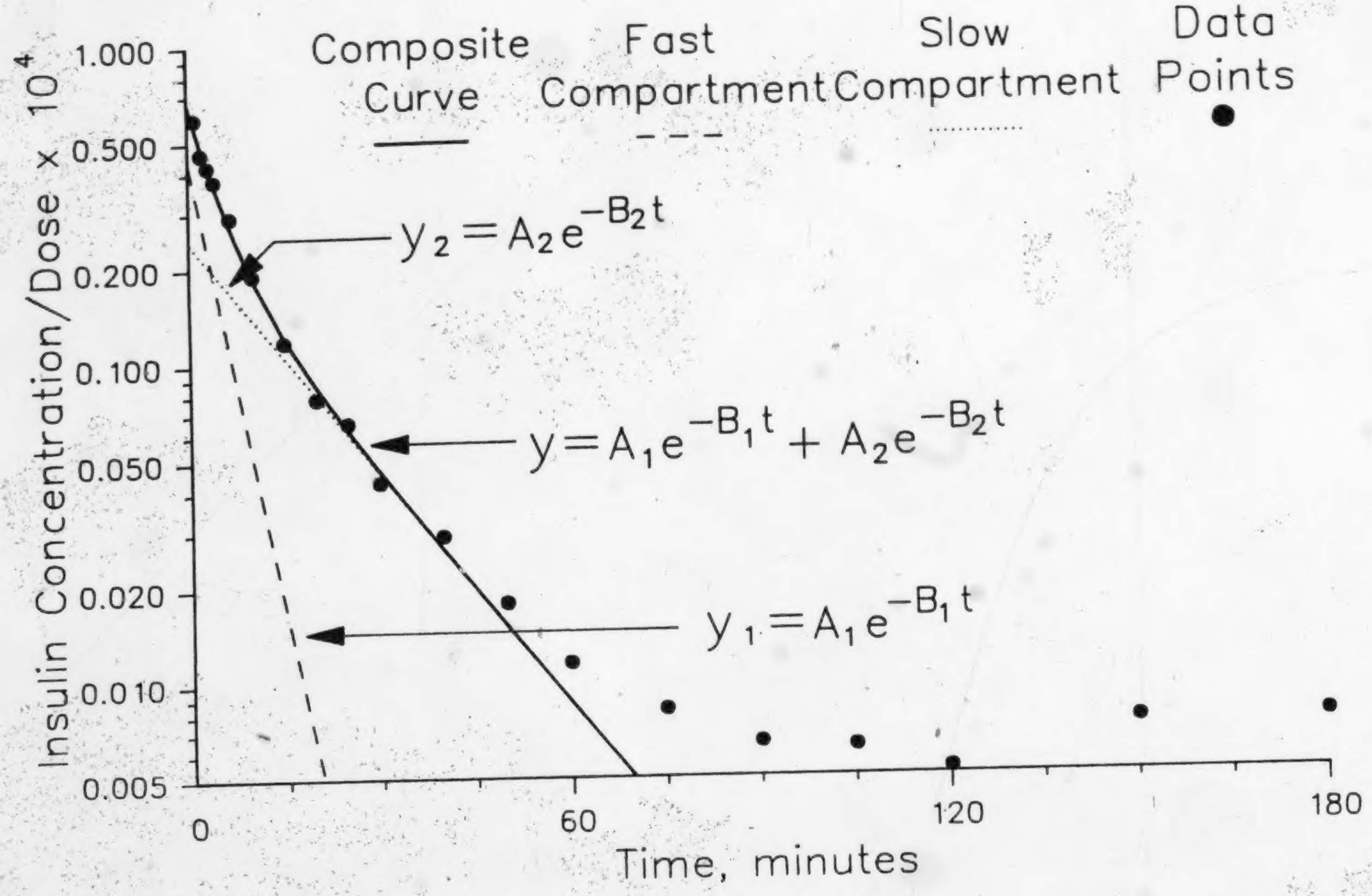

Insulin Concentration/Dose x 10^4
1.000
0.500
0.200
0.100
0.050
0.020
0.010
0.005
0
60
120
180
Time, minutes
Composite Curve
Fast Compartment
Slow Compartment
Data Points
$y_2 = A_2 e^{-B_2 t}$
$y = A_1 e^{-B_1 t} + A_2 e^{-B_2 t}$
$y_1 = A_1 e^{-B_1 t}$

FIGURE III-2: Effect of insulin injection on plasma glucose concentration (mg mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. The pooled SEM was 0.013 for the warm and 0.013 for the cold.

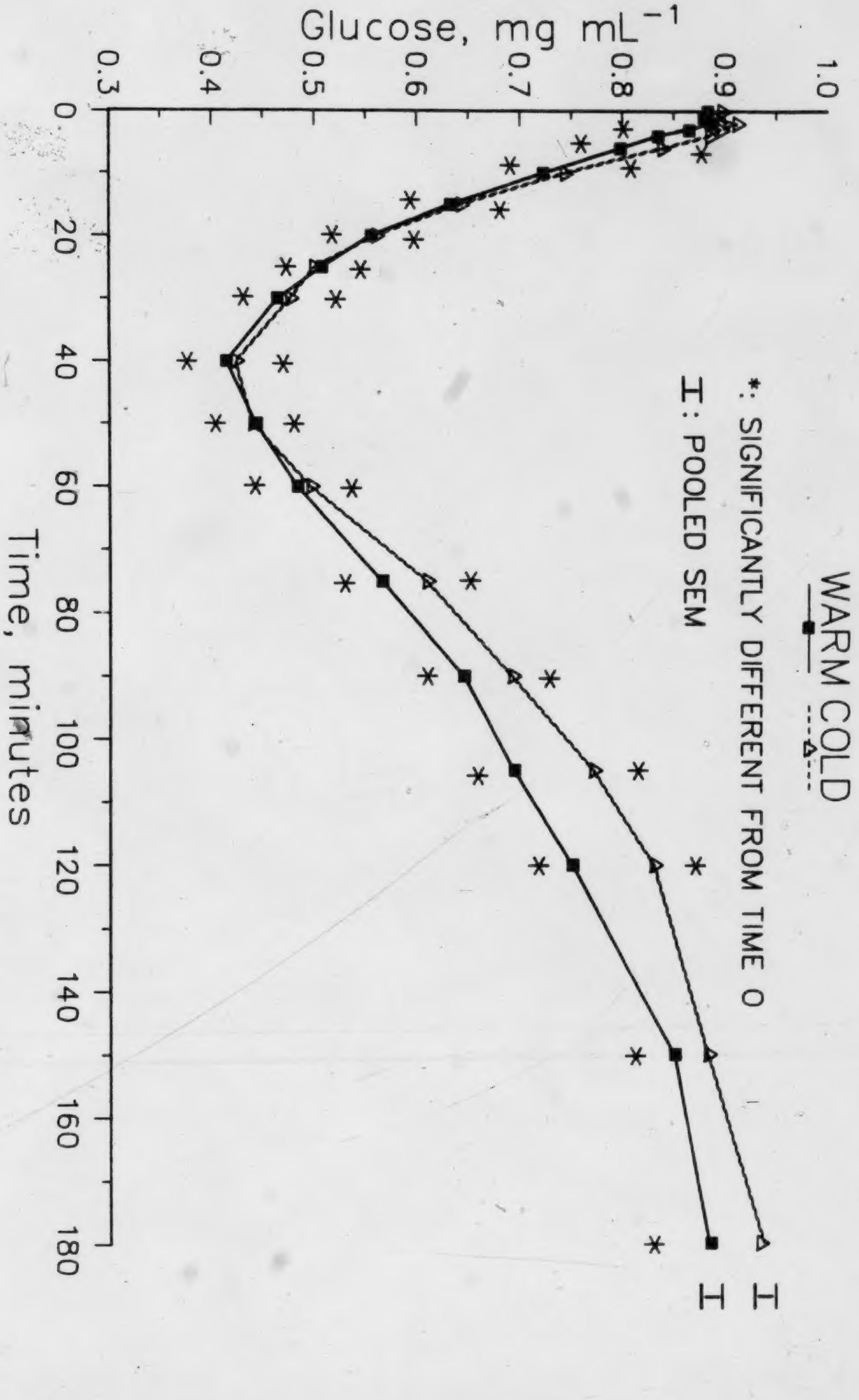

Glucose, mg mL⁻¹
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
20
40
60
80
100
120
140
160
180
Time, minutes
WARM
COLD
*: SIGNIFICANTLY DIFFERENT FROM TIME 0
I: POOLED SEM

FIGURE III-3: Effect of insulin injection on plasma glucagon concentration (pg mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. The pooled SEM was 30.3 for the warm and 26.8 for the cold.

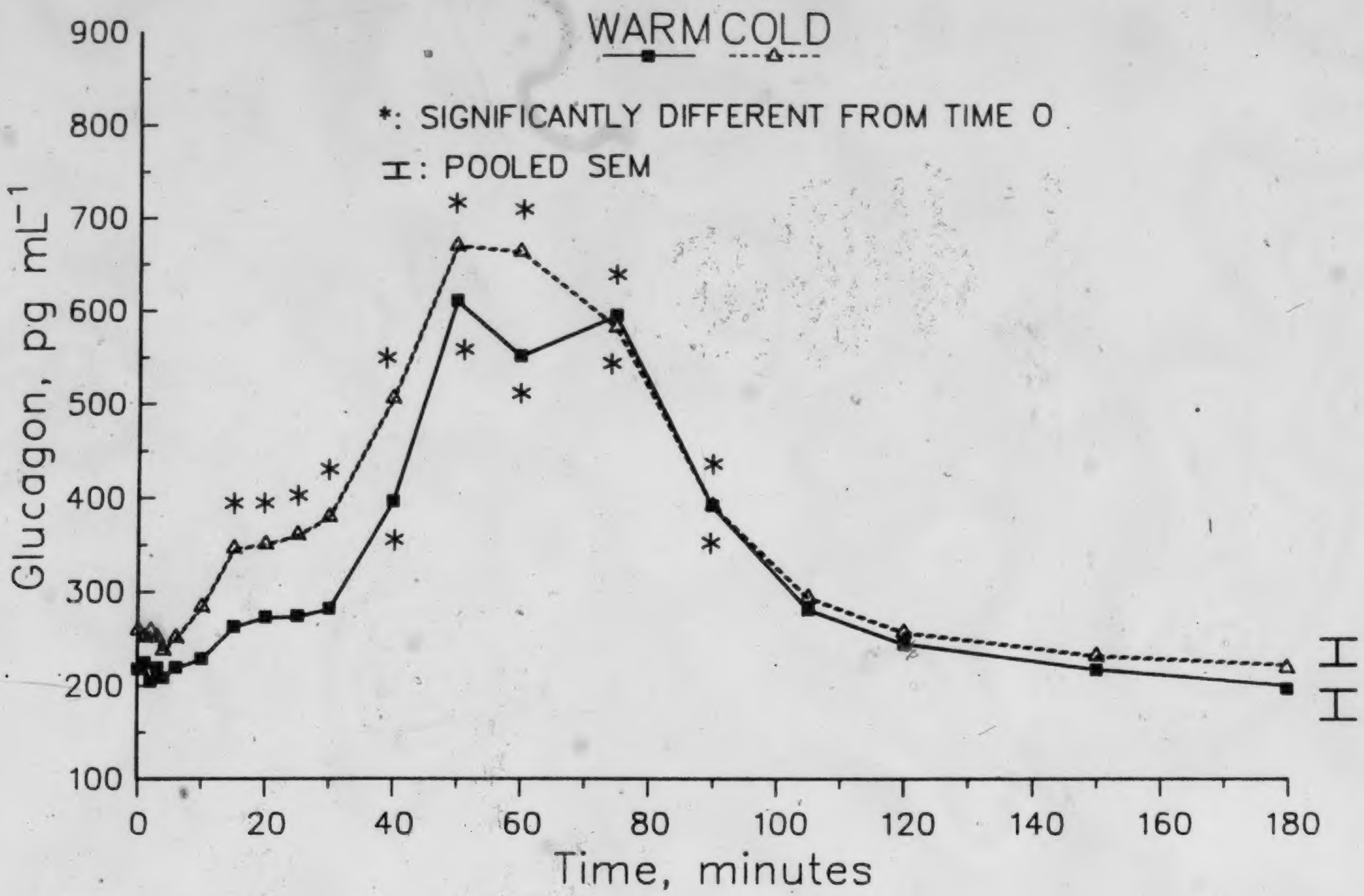

WARM
COLD
*: SIGNIFICANTLY DIFFERENT FROM TIME O
⌶: POOLED SEM
Glucagon, pg mL^{-1}
900
800
700
600
500
400
300
200
100
0
20
40
60
80
100
120
140
160
180
Time, minutes

FIGURE III-4: Effect of insulin injection on plasma growth hormone concentration (ng mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. The pooled SEM was 0.358 for the warm and 0.373 for the cold.

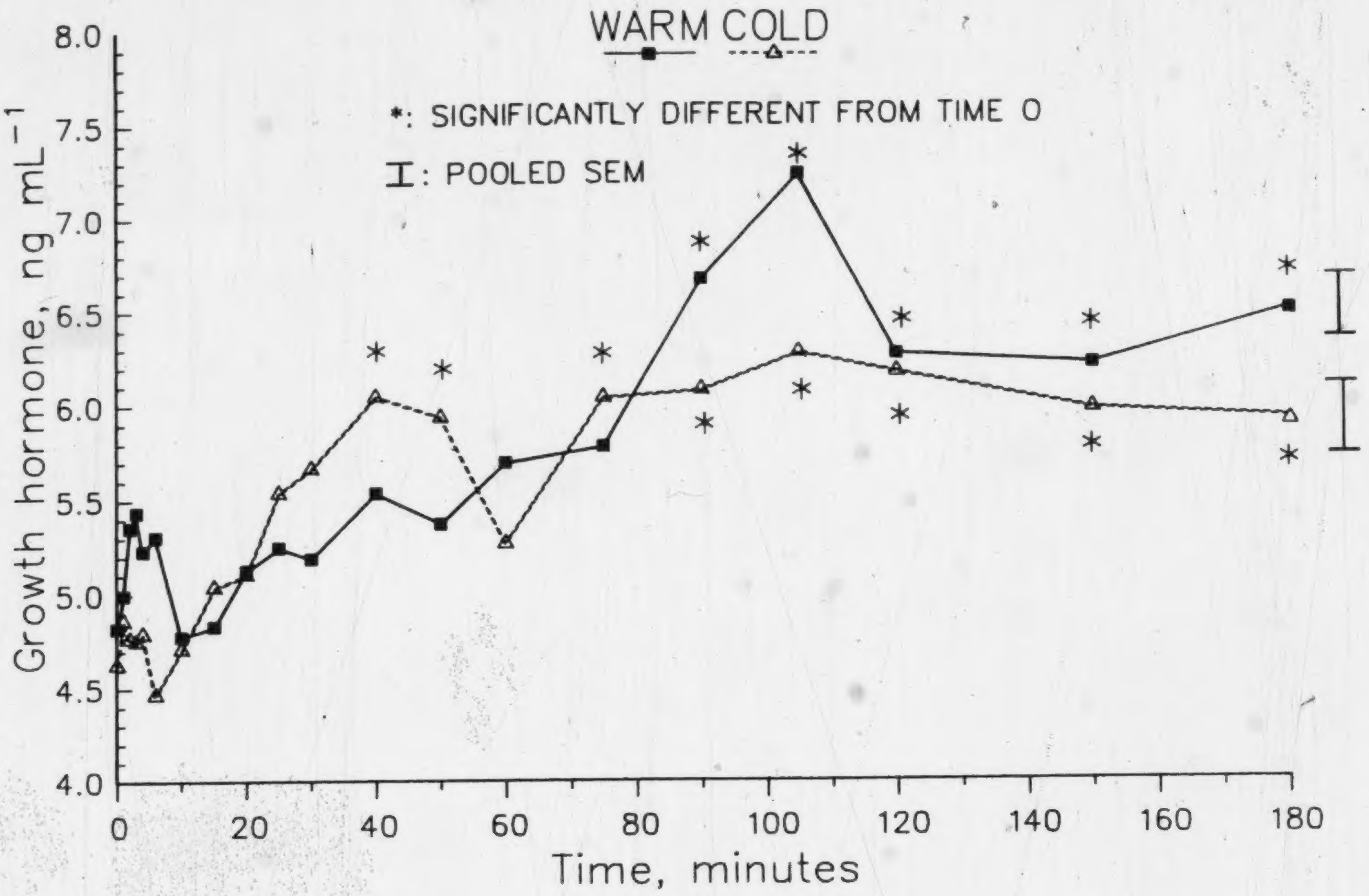

WARM
COLD
*: SIGNIFICANTLY DIFFERENT FROM TIME 0
I: POOLED SEM
Growth hormone, ng mL^{-1}
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
0
20
40
60
80
100
120
140
160
180
Time, minutes

FIGURE III-5: Effect of growth hormone injection on plasma glucose concentration (mg mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. The pooled SEM was 0.012 for the warm and 0.012 for the cold. (Only data from Period 2 shown).

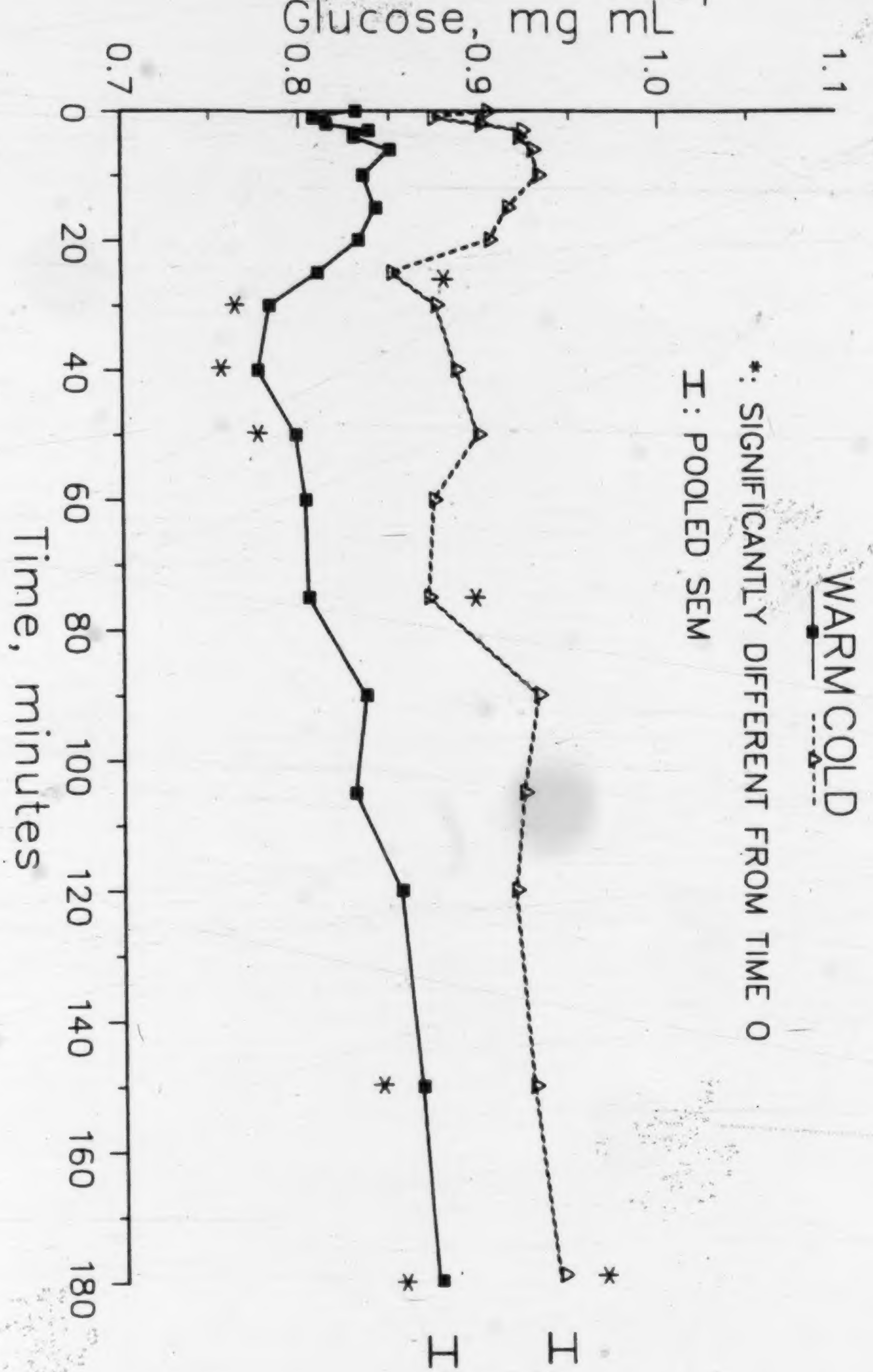

WARM
COLD
*: SIGNIFICANTLY DIFFERENT FROM TIME 0
I: POOLED SEM
Glucose, mg mL^{-1}
0.7
0.8
0.9
1.0
1.1
Time, minutes
0
20
40
60
80
100
120
140
160
180

FIGURE III-6: Effect of growth hormone injection on plasma insulin concentration (ng mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. The pooled SEM was 0.186 for the warm and 0.320 for the cold. (Only data from Period 2 shown).

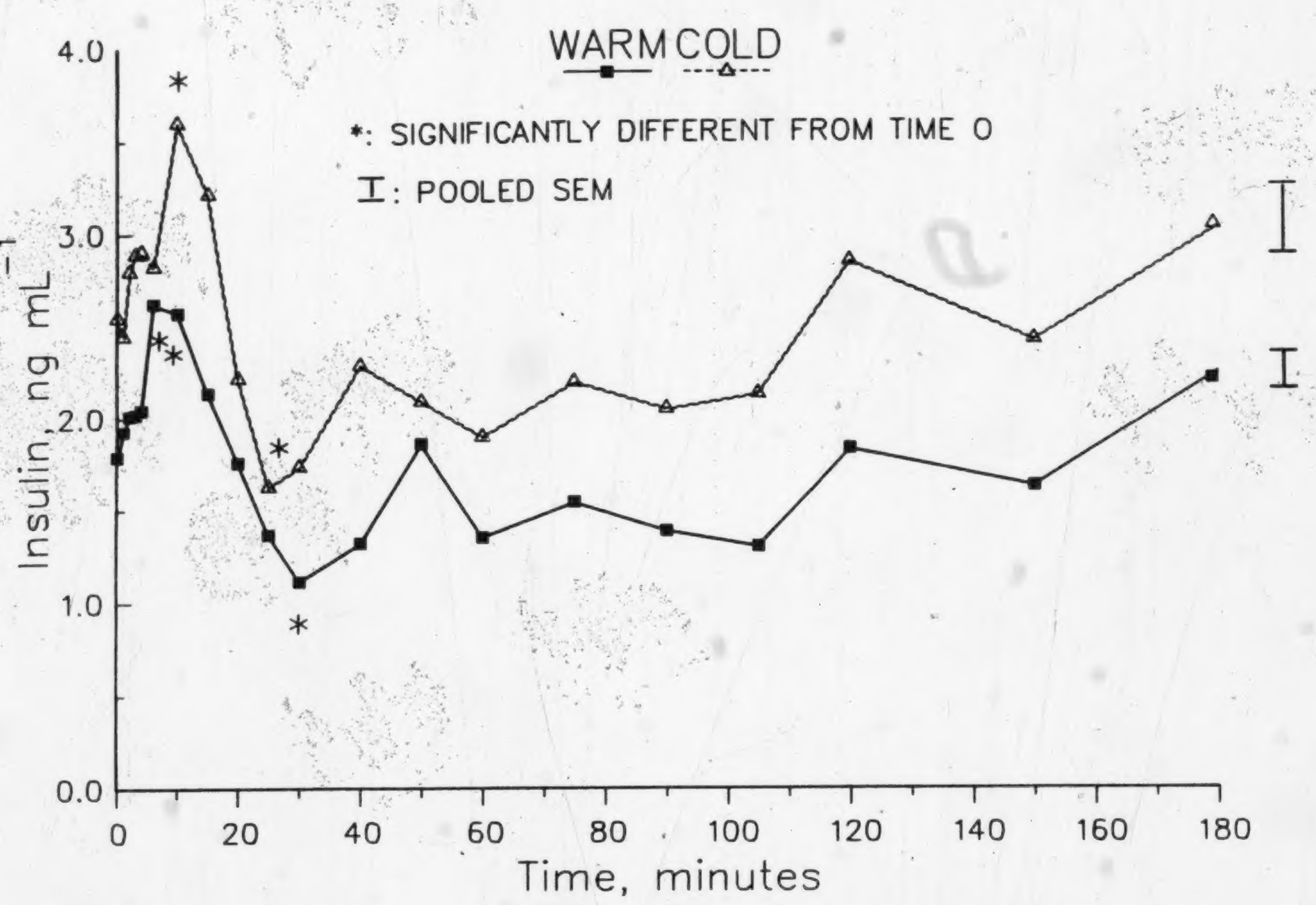

WARM
COLD
*: SIGNIFICANTLY DIFFERENT FROM TIME 0
I: POOLED SEM
Insulin, ng mL^{-1}
0.0
1.0
2.0
3.0
4.0
Time, minutes
0
20
40
60
80
100
120
140
160
180
*

FIGURE III-7: Effect of growth hormone injections at time 0 hours and time 24 hours on serum insulin-like growth factor I concentration (ng mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. The pooled SEM was 16.1 for the warm and 13.8 for the cold. (Only data from Period 2 shown).

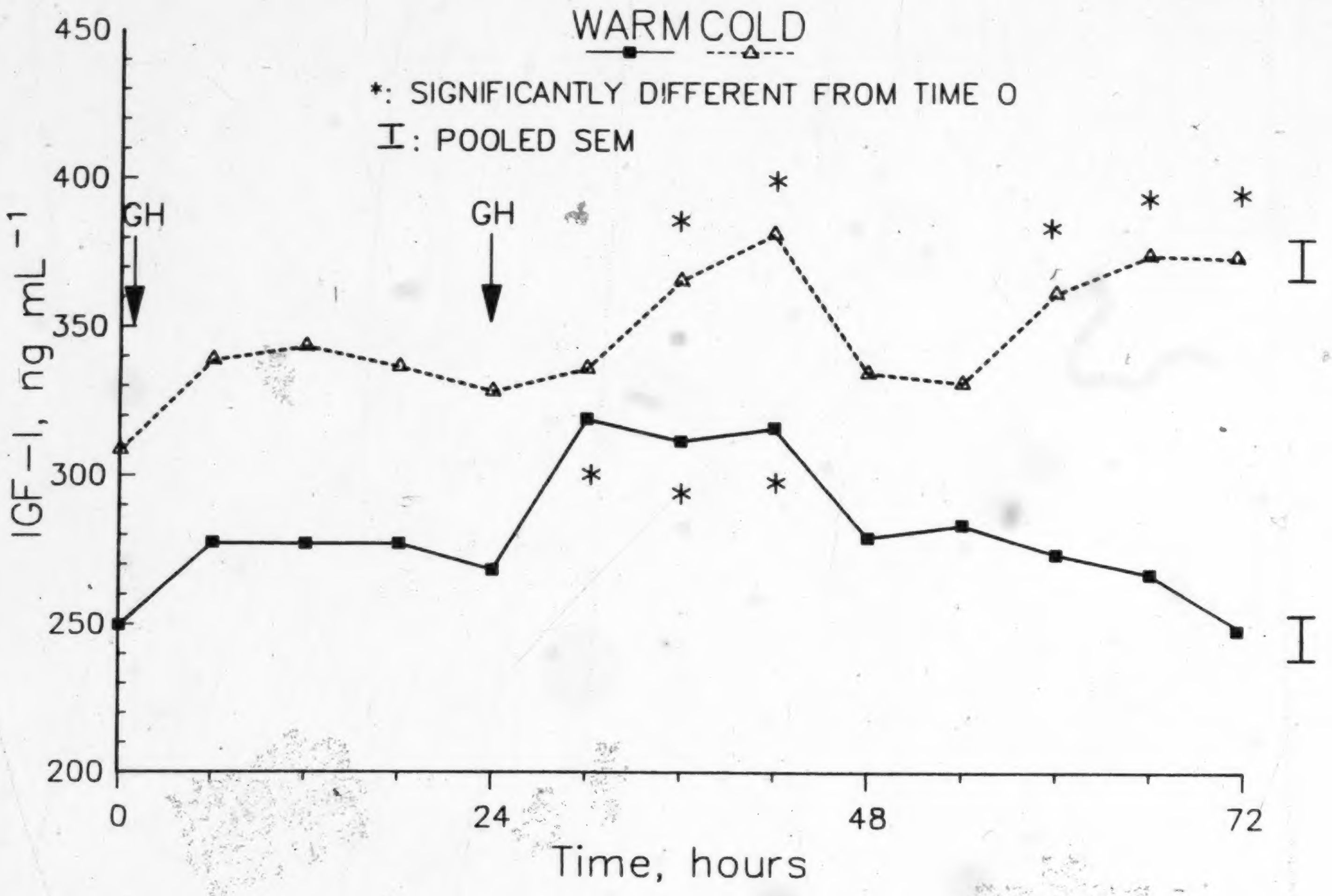
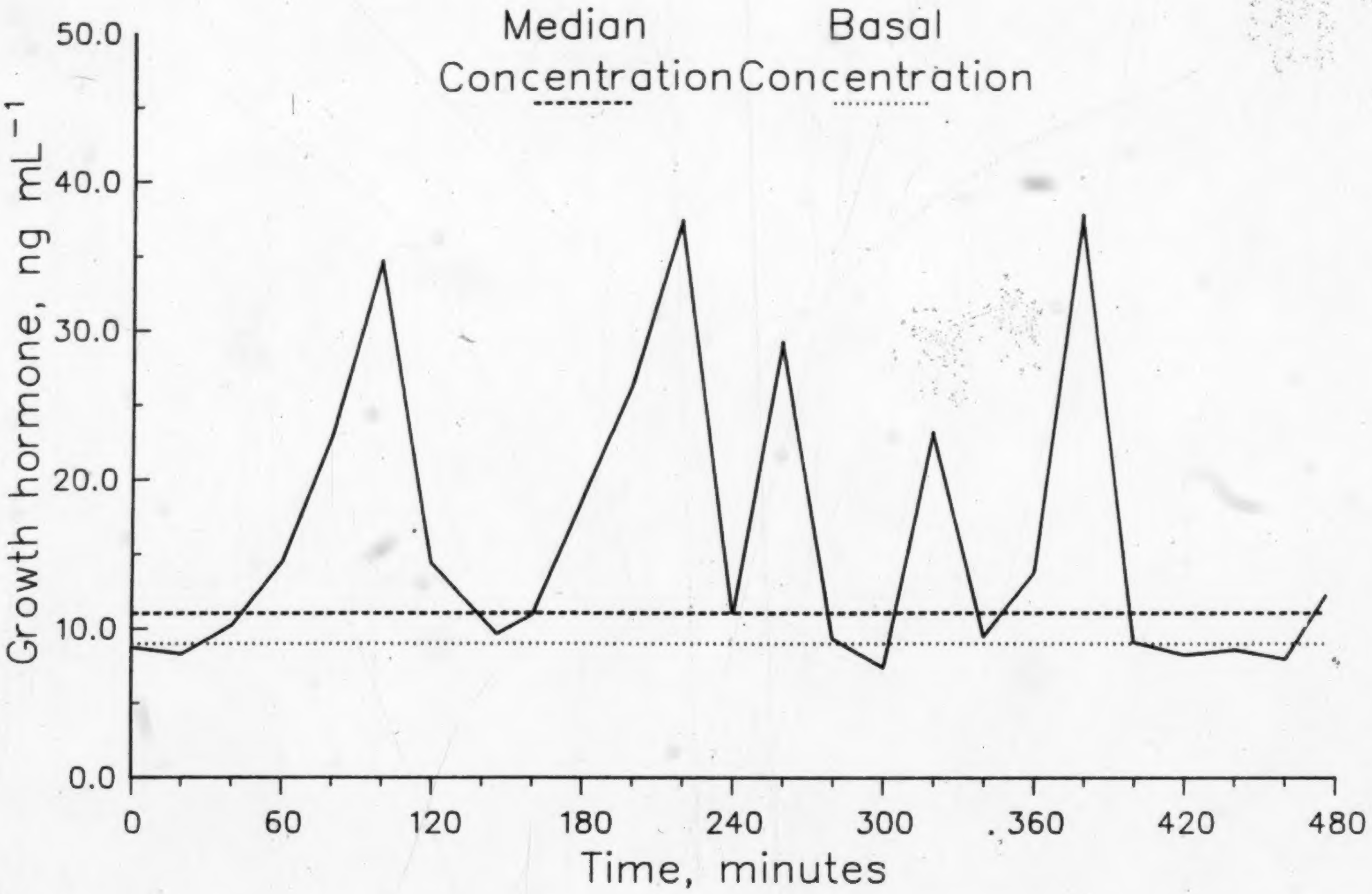

WARM
COLD
*: SIGNIFICANTLY DIFFERENT FROM TIME 0
I: POOLED SEM
GH
GH
IGF-I, ng mL⁻¹
450
400
350
300
250
200
0
24
48
72
Time, hours

FIGURE III-8: An example of pulsatile growth hormone release during an 8 hour continuous infusion of ^{3}H-phenylalanine in calf 832 in the W72 group. Plasma samples were taken every 20 minutes.


Median Concentration
Basal Concentration
Growth hormone, ng mL^{-1}
50.0
40.0
30.0
20.0
10.0
0.0
0
60
120
180
240
300
360
420
480
Time, minutes

LITERATURE CITED

Bassett, J.M., and A.L.C. Wallace. 1966. Short-term effects of ovine growth hormone on plasma glucose, free fatty acids and ketones in sheep. Metab. Clin. Exp. 15: 933-944.

Bauman, D.E., and S.N. M^{c}Cutcheon. 1985. The effects of growth hormone and prolactin on metabolism. In: Control of Digestion and Metabolism in Ruminants, L.P. Milligan, W.L. Grovum and A. Dobson, eds., pp. 436-455. Prentice-Hall, Englewood Cliffs, USA.

Blom, A.K., Hove, K., and J.J. Nedkvitne. 1976. Plasma insulin and growth hormone concentrations in pregnant sheep II: post-absorptive levels in mid- and late pregnancy. Acta Endocrin. 82: 553-560.

Breier, B.H., Bass, J.J., Butler, J.H., and P.D. Gluckman. 1986. The somatotrophic axis in young steers: influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor 1. J. Endocr. 111: 209-215.

Brockman, R.P., and B. Laarveld. 1986. Hormonal regulation of metabolism in ruminants: a review. Livest. Prod. Sci. 14: 313-334.

Christopherson, R.J., Thompson, J.R., Hammond, V.A., and G.A. Hills. 1978. Effects of thyroid status on plasma adrenaline and noradrenaline concentrations in sheep during acute and chronic cold exposure. Can. J. Physiol. Pharmacol. 56: 490-496.

Dauncey, M.J., Shakespear, R.A., Rudd, B.T., and D.L. Ingram. 1990. Variations in somatomedin-C/insulin-like growth factor-I associated with environmental temperature and nutrition. Horm. Metab. Res. 22: 261-264.

De Boer, G., and J.J. Kennelly. 1989. Effect of somatotropin injection and dietary protein concentration on milk yield, and kinetics of hormones in dairy cows. J. Dairy Sci. 72: 419-428.

De Boer, G., Trenkle, A., and J.W. Young. 1986. Secretion and clearance rates of glucagon in dairy cows. J. Dairy Sci. 69: 721-733.

Elsasser, T.H., Rumsey, T.S., and A.C. Hammond. 1989. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. J. Anim. Sci. 67: 128-141.

Evans, W.S., Faria, A.C.S., Christiansen, E., Ho, K.Y., Weiss, J., Rogol, A.D., Johnson, M.L., Blizzard, R.M., Veldhuis, J.D., and M.O. Thorner. 1987. Impact of intensive venous sampling on characterization of pulsatile GH release. Am. J. Physiol. 252: E549-E556.

Fuller, M.F. 1965. The effect of environmental temperature on the nitrogen metabolism and growth of the young pig. Br. J. Nutr. 19: 531-546.

Glimm, D.R., Baracos, V.E., and J.J. Kennelly. 1988. Effect of bovine somatotropin on the distribution of immunoreactive insulin-like growth factor-I in lactating bovine mammary tissue. J Dairy Sci. 71: 2923-2935.

Godden, P.M.M., and T.E.C. Weekes. 1984. Influence of chronic thyroxine treatment on plasma hormone and metabolite concentrations and on responses to insulin, glucagon and thyrotrophin releasing hormone in adult sheep. Horm. Metab. Res. 16: 354-358.

Harris, V.A., Faloona, G.R., and R.H. Unger. 1979. Glucagon. In: Methods of Hormone Radioimmunoassay, 2nd edition, B.M. Jaffe and H.R. Behrman, eds., pp. 643-656. Academic Press, New York.

Kipnis, D.M., Hertelendy, F., and L.J. Machlin. 1969. Studies of growth hormone secretion. In: Progress in Endocrinology, C. Gual and F.J.G. Ebling, eds., pp. 601-609. Excerpta Medica Foundation International Congress Series, No. 184. Excerpta Medica Foundation, Amsterdam.

Machlin, L.J., Takahashi, Y., Horino, M., Hertelendy, F., Gordon, R.S., and D. Kipnis. 1967. Regulation of growth hormone secretion in non-primate species. In: Excerpta Medica International Congress Series No. 142, A. Pecile, E.E. Müller, and F.C. Greenwood, eds, p. 16 (Abstr.). Excerpta Medica Foundation, Amsterdam.

McKay, D.G. 1974. Utilization of energy substrates by ruminants exposed to cold. Ph.D. Thesis. University of Alberta, Edmonton, Canada.

National Research Council. 1978. Nutrient Requirements of Dairy Cattle, 5th edition. National Academy of Sciences, Washington, D.C.
Newsholme, E.A. 1987. Substrate cycles and energy metabolism: Their biochemical, biological, physiological and pathological importance. In: Energy Metabolism of Farm Animals, P.W. Moe, H.F. Tyrrell, and P.J. Reynolds, eds. EAAP Publication No. 32. Rowman and Littlefield,

Okada, Y., Miyai, K., Iwatsubo, H., and Y. Kumahara. 1970. Human growth hormone secretion in normal adult subjects during and after exposure to cold. J. Clin. Endocr. 30: 393-395.

Olsen, J.D., and A. Trenkle. 1973. Exposure of cattle to controlled subzero temperature: growth hormone, glucose, and free fatty acid concentrations in plasma. Am. J. Vet. Res. 34: 747-751.

SAS Institute Inc. 1985. SAS User's Guide: Statistics, Version 5 edition. SAS Institute, Inc., Cary, USA.

Sasaki, Y., Takahashi, H., Aso, H., Ohneda, A., and T.E.C. Weekes. 1982. Effects of cold exposure on insulin and glucagon secretion in sheep. Endocrinology 111: 2070-2076.

Shipley, R.A., and R.E. Clark. 1972. Tracer Methods for In-Vivo Kinetics: Theory and Applications. Academic Press, New York.

SPSS Inc. 1988. SPSS-X User's Guide, 3rd edition. SPSS Inc., Chicago, USA.

Steele, R.G.D., and J.H. Torrie. 1980. Principles and prodedures of statistics. McGraw-Hill Book Co., New York, USA.

Takahashi, H., Murata, H., and H. Matsumoto. 1984. Insulin, glucagon and 11-OHCS responses to arginine in calves exposed to cold. Can. J. Anim. Sci. 64(Suppl.): 267-268.

Trenkle, A. 1971. Influence of blood glucose levels on growth hormone secretion in sheep. Proc. Soc. Exp. Med. Biol. 136: 51-55.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6: 24.

Vallerand, A.L., Lupien, J., and L.J. Bukowiecki. 1983. Interactions of cold exposure and starvation on glucose tolerance and insulin response. Am. J. Physiol. 245: E575-E581.

Weekes, T.E.C., Sasaki, Y., and T. Tsuda. 1983. Enhanced responsiveness to insulin in sheep exposed to cold. Am. J. Physiol. 244: E335-E345.

Young, V.R. 1980. Hormonal control of protein metabolism, with particular reference to body protein gain. In: Protein Deposition in Animals, P.J. Buttery and D.B. Lindsay, eds., pp. 167-191. Butterworths, London.

Zapf, J., and E.R. Froesch. 1986. Insulin-like growth factors/somatomedins: Structure, secretion, biological actions and physiological role. Horm. Res. 24: 121-130.

CHAPTER IV

GENERAL DISCUSSION AND CONCLUSIONS

Introduction

Protein turnover is a complex process with a host of regulators. In mature animals, the rate of protein synthesis normally balances the rate of protein degradation. A certain amount of protein synthesis and degradation is necessary to maintain the structural integrity of the body, even in growing animals (Reeds and Lobley, 1980). In animal production, we depend on the rate of protein synthesis exceeding that of protein degradation in growing animals so that protein deposition occurs. Only a small increment in either rate can result in large differences in protein retention over time (Reeds and Fuller, 1983). Information regarding the direction of changes in protein synthesis and degradation induced by various regulators of protein turnover is, therefore, of importance to animal production.

In this thesis, I attempted to determine the effect of cold-adaptation on protein synthesis in growing cattle and to test the hypothesis that elevated protein synthesis contributes to cold-induced thermogenesis (Yousef and Chaffee, 1970). Although protein degradation is also an important component of protein turnover, reliable methods were not available to estimate protein degradation in any tissue except muscle. In view of the many hormones that regulate protein turnover (Millward *et al.*, 1983), possible alterations in hormone concentrations or kinetic parameters in response to cold-adaptation were also investigated.

These studies demonstrated that whole-body protein retention and fractional synthesis rates of protein in muscle and skin were depressed in cold-adapted calves on restricted feed intake. In the cold environment, muscle and skin protein synthesis were positively related to increased protein intake resulting from increased feed intake. In addition, muscle protein breakdown appeared to be elevated in the cold, with the net result that muscle protein gain was reduced. Since meat production is dependent on muscle protein deposition, these alterations in muscle protein metabolism indicate an important source of production loss in cold-adapted animals. Increased concentrations of catabolic and gluconeogenic hormones such as thyroxine, triiodothyronine, and glucagon in cold-adapted heifers suggested that the endocrine system may have directed a shift from anabolism to catabolism of amino acids.

Effects of Cold-adaptation on Nitrogen Retention

Alterations in nitrogen retention as a result of cold-adaptation have been reported for ruminants. In lambs nitrogen retention was reduced at lower temperatures despite increased voluntary feed intake (Ames and Brink, 1977). Nitrogen retention was depressed by 50 % and urinary nitrogen excretion elevated in yearling wethers exposed to 0.8°C and fed the same amount as controls at 17.7°C (Westra and Christopherson, 1976). Similar results were obtained in this thesis for the C72 group compared with the W72 group (Chapter II and Appendix 1). However, cold-adapted calves in the C90 group were able to maintain nitrogen retention at a level similar to that of calves in the warm as a result of a 20% increase in feed intake.

Feed intake obviously modulates the effects of cold-adaptation on nitrogen retention. Since higher feed intake in the cold was necessary to maintain nitrogen retention at a level similar to that in the warm, it is likely that the linear curve relating metabolizable energy intake to nitrogen retention (Campbell, 1988) was shifted to the right as a result of cold-adaptation. In addition, if lower critical temperature was higher at low intake (Mount, 1980), a greater shift in the curve would occur at low intake, thus altering the slope. If protein requirements are modified by cold-adaptation, diet protein content may also have an effect on the position or slope of the curve. It is possible that diets of a lower protein:energy ratio could be offered to cold-adapted ruminants without adversely affecting nitrogen retention since cold-adaptation improved the ratio of g non-ammonia nitrogen digested to kg organic matter digested intestinally in sheep (Kennedy *et al.*, 1986). Ames *et al.* (1980) also showed that if a portion of dietary protein were replaced with an energy source, the remaining protein was used for gain more efficiently in cold-adapted cattle and sheep fed *ad libitum*. Therefore, offering diets varying in protein and energy content could indicate the optimal protein:energy ratio and feed intake to maximize nitrogen retention in cold-adapted animals.

Effects of Cold-adaptation on Amino Acid Catabolism

As a proportion of flux, glucose and palmitate were oxidized more extensively in ewes conditioned to -2°C compared with 18°C, despite a 20% higher feed intake (M^{c}Kay, 1974). Although phenylalanine catabolism was not determined in Chapter II, it is likely that the fraction of amino acid flux oxidized would be similarly augmented in cold-adapted calves, as indicated by elevated plasma phenylalanine flux in the cold without a concomitant increase in protein synthesis. This hypothesis is supported by results demonstrating increased leucine catabolism during an acute cold stress of 10°C in man (Goodenough *et al.*, 1982) and increased tyrosine catabolism in pigs adapted to 10°C Lindsay *et al.*, 1988). In addition, Lindsay *et al.* (1988) showed a strong positive relationship between tyrosine flux and tyrosine catabolism, which may connote more rapid phenylalanine oxidation in cold-adapted calves. Carbon dioxide production from labeled amino acids in warm and cold environments could provide estimates of oxidation rates. Amino acid flux corrected for oxidation would also present a more accurate picture of whole-body protein synthesis.

Amino acids can be used as an energy source either via complete oxidation or gluconeogenesis. Use of amino acids as gluconeogenic precursors to provide substrate for glucose-dependent tissues would likely be enhanced following cold-adaptation. However, Lindsay (1982) argued that use of amino acids for gluconeogenesis is limited in ruminants. Determination of glucose production from a variety of amino acids in warm- and cold-adapted animals is necessary to resolve this discrepancy.

Use of amino acids as an energy source in a cold environment will limit their use for protein synthesis. This does not agree with the contention of Lindsay (1982) that altering amino acid catabolism is unlikely to affect rates of protein synthesis. However, his conclusions were mainly drawn from studies involving animals in a thermoneutral environment. They do not take into account the possibility that under conditions such as cold stress where demands for energy or glucose have escalated, amino acid catabolism could limit protein synthesis (MacRae and Reeds, 1980).

Effects of Cold-adaptation on Protein Synthesis

Alterations in protein turnover following exposure to a cold environment have been reported in a variety of species, with disparate results. In chicks fed *ad libitum* and reared at 22°C for 7 days, the fractional synthesis rate of protein in the whole body, but not in the liver, was increased compared with 30°C (Aoyagi *et al.*, 1988). However, in the same experiment, thermogenesis from enhanced protein synthesis only accounted for 1.4 % of the increment in heat production at the lower ambient temperature. In rainbow trout, a poikilotherm, the fractional synthesis rates of protein in liver and the digestive tract were depressed at 10°C compared with 18°C, although muscle and whole-body protein synthesis were unaffected (Fauconneau *et al.*, 1981; Fauconneau and Arnal, 1985). In rats, both the fractional rates of synthesis and degradation of protein in muscle were elevated along with feed intake during a 14 day acclimation to 4°C (Brown *et al.*, 1984). These results were not supported by findings of M^{c}Allister (1987) that both synthesis and degradation were decreased in skeletal muscle but increased in liver and heart in rats fed *ad libitum* and adapted to 4°C for 20 days. In Chapter II, only the fractional synthesis rates of protein in muscle and skin were influenced by cold-adaptation, and then only in calves on restricted intake. It is difficult to summarize the effect of environmental temperature on protein turnover, since it seems to vary within as well as between species. The diverse methods used to approach the question may have affected the results obtained. It is also obvious that effects due to cold-adaptation must be discerned from those due to increased feed intake, which was the reason that calves in the cold in Chapter II were fed at two different levels of intake. Further studies concerning the effect of cold on protein turnover should incorporate different levels of feed intake into the experimental design.

The observation that fractional synthesis rates of protein in muscle and skin were depressed only in the C72 group implies that feed intake was not adequate to allow protein synthesis to continue at the same rate as in the other two groups. In addition, the lack of a general reduction in protein synthesis in all tissues suggests that muscle and skin have a lower priority for the reduced nutrient supply than other tissues (Buttery and Bryan, 1986). Why would muscle and skin have a lower priority than other tissues? They both have important functions in the body; muscle is required for movement and skin forms a protective and insulative barrier against the external environment. In addition to the replacement of damaged proteins, protein turnover in these two tissues is related to their function. Muscle protein synthesis is necessary for muscle growth and maintenance of muscle mass for locomotion, and skin protein synthesis is necessary to replace sloughed epithelial cells and produce specialized proteins which make up hair, wool, and nails or hooves.

Scant information is available in the literature regarding rates and regulation of protein turnover in skin. Data available for the fractional synthesis rate of protein in skin as well as the contribution of skin protein synthesis to whole-body protein synthesis are summarized in Table IV-1. It is apparent that skin accounts for a significant proportion of whole-body protein synthesis. The apparently anomalous value observed for the fractional synthesis rate of protein in skin from rats (Preedy *et al.*, 1983) only applies to skin protein soluble in 0.3 M NaOH. The authors of the paper estimated that if insoluble protein were included in the calculations along with soluble protein, the fractional synthesis rate would fall to 13 % day^{-1}, similar to values for other species.

Nutritional state and substrate availability may regulate skin protein synthesis. Allison and Wannemacher (1965) contended that skin represents a significant labile protein reserve during conditions of nutritional stress. According to their definition, a labile protein reserve includes tissue proteins that can be reversibly depleted when necessary in order to contribute free amino acids to body pools. They also suggested that nutritional stress would depress skin protein synthesis. This is supported by results showing a 26 % reduction in the fractional synthesis rate of alkaline-soluble skin protein following a two-day fast (Preedy *et al.*, 1983). In the same experiment, alkaline-insoluble protein, comprising collagen, keratin and elastin, had such a slow fractional synthesis rate that no detectable label was incorporated into that fraction. Apparently, protein synthesis in the alkaline-soluble fraction could be altered without a concomitant change in the structural proteins of the skin. Such a dual response would allow a reduction in skin protein synthesis during conditions where nutrient supply is limited without jeopardizing its functional integrity as a barrier.

The degree of nutritional stress will determine if skin protein degradation is increased or decreased. If only energy is limited, skin protein degradation could decrease along with synthesis. This overall reduction in protein turnover would act as an energy-sparing mechanism. However, if both energy and protein were limited, skin could serve as a labile protein reserve along with muscle, supplying amino acids to tissues having a higher priority for nutrients. Although protein degradation and protein synthesis usually change in the same direction (Reeds and Fuller, 1983), muscle protein degradation appeared to increase in the cold-adapted calves in Chapter II, as indicated by elevated urinary N^{τ}-methylhistidine excretion, without a concomitant increase in protein synthesis. Since both energy and protein were likely limited in the C72 group of calves, a similar elevation in skin protein degradation would be expected.

Skin protein turnover may be an important contributor to whole-body protein metabolism, especially during conditions of nutritional or environmental stress. Therefore, further information is required regarding the magnitude and regulation of skin protein turnover. Since the alkaline-soluble and alkaline-insoluble fractions of skin protein appear to be regulated independently, experiments should attempt to determine treatment effects on each fraction. The continuous infusion method may be more appropriate for measuring the fractional synthesis rate of protein in skin, since the prolonged infusion period could allow estimation of synthesis rates in both fractions of skin protein. The hypothesis that skin may not be as sensitive to nutritional stress as muscle (Preedy *et al.*, 1983) should be explored. Effects of protein and energy intake on protein degradation and amino acid release from skin could be compared with muscle from measurements of arterio-venous differences in amino acid concentrations across a section of each tissue, along with estimations of blood flow. One such preparation for the skin of the abdominal flank has been used by Harris and Lobley (1989) to measure skin metabolism. Alternatively, changes in the protein content of the alkaline-soluble and alkaline-insoluble fractions of skin protein could indicate changes in protein degradation. The possibility that in cold-adapted animals reduced skin temperatures (Young, 1975) may directly affect skin protein synthesis should be examined, since Baracos *et al.* (1984) demonstrated *in vitro* protein synthesis was 25 % lower in muscles incubated at 33°C compared with 39°C. Finally, although wool and hair growth were not affected by cold-adaptation, hair shedding was reduced in cold-exposed animals (Webster *et al.*, 1969; Webster *et al.*, 1970), possibly altering the contribution of hair or wool growth to skin protein turnover.

Effects of Cold-adaptation on the Endocrine System

With the observed effects of cold-adaptation on fractional synthesis rates of protein in muscle and skin from calves on restricted intake, it was expected that hormonal alterations would also be evident in Chapter III. Likely, the high variability within groups of calves masked possible changes. However, endocrine responses to cold-adaptation observed in the heifers in Chapter III may be correlated with changes in protein synthesis, since a variety of hormones are regulators of both protein synthesis and degradation. Since the heifers were fed hay *ad libitum*, it may be more accurate to compare the cold-adapted heifers with the C90 group of calves.

As an anabolic hormone, insulin is considered to promote protein synthesis (Young, 1980). In wether lambs receiving a constant intravenous infusion of insulin and glucose, plasma α-amino nitrogen and urea concentrations were reduced, although urinary and fecal nitrogen excretion were unaffected (Sumner and Weekes, 1983). If cattle were infused with glucose along with an injection of insulin, plasma branched-chain amino acid concentrations decreased (Prior and Smith, 1983). These results imply that insulin could lower plasma amino acid levels either by promoting tissue uptake of amino acids and protein synthesis, or by retarding proteolysis and subsequent branched-chain amino acid release. In sheep, a concurrent infusion of insulin and glucose had no effect on either the *in vitro* or *in vivo* fractional synthesis rates of protein in skeletal muscle (Early *et al*., 1988). The lack of response to insulin in ruminant muscle may reflect a species difference, as insulin increased muscle protein synthesis in rats both *in vitro* (Fulks *et al*., 1975) and *in vivo* (Reeds *et al*., 1985). It may also be due to the fact that the sheep were fed, since Garlick *et al*. (1983) reported that insulin infusion increased muscle protein synthesis in post-absorptive but not fed rats. Although the above results suggest that insulin regulates amino acid metabolism in ruminants, a direct effect on protein synthesis has yet to be demonstrated.

Although muscle protein synthesis in ruminants may be unresponsive to insulin in fed animals under thermoneutral conditions, Weekes *et al.* (1983) reported that whole-body glucose utilization exhibited an enhanced responsiveness to insulin following cold-adaptation, and suggested muscle as a likely site of action. Perhaps in the C90 group, increased responsiveness of muscle protein synthesis to insulin prevented a fall in protein synthesis following cold-adaptation. Conversely, elevated insulin concentrations in the cold-adapted animals may have decreased receptor binding affinity and/or concentration, resulting in a reduced sensitivity of protein synthesis to insulin (Weekes *et al.*, 1983). Therefore, even at higher insulin concentrations during cold-adaptation, protein synthesis would not change. Very few studies have examined insulin receptor affinity or concentrations in ruminant tissues; information is especially lacking for muscle. In addition, dose-response curves in muscle for the two main actions of insulin, glucose utilization and protein synthesis, may be quite different (Buttery, 1983). A smaller increment in insulin concentration may be necessary to increase glucose utilization than protein synthesis. It would be informative to determine the effect of cold-adaptation on insulin cell surface receptor concentration and binding affinity in a variety of tissues, especially muscle and skin. Probably, cold-adaptation has differential effects in muscle on the insulin dose-response curves to glucose utilization and protein synthesis.

Since IGF-I production in peripheral tissues is influenced by growth hormone (Young, 1980), the elevated IGF-I concentrations in the cold-adapted heifers without a simultaneous increase in growth hormone concentration or alteration in kinetic parameters was unexpected. However, the higher *ad libitum* feed intake of the cold-adapted heifers may have been a factor, since steers on a high plane of nutrition had higher plasma IGF-I concentrations compared with steers on a low plane of nutrition, even though there were no differences in plasma growth hormone concentrations (Elsasser *et al.*, 1989). IGF-I is considered to be a protein anabolic hormone, and would therefore be expected to increase protein synthesis in muscle (Young, 1980). However, it may be that in the cold-adapted heifers, the insulin-like actions of IGF-I, such as enhanced glucose utilization, are more prominent rather than its protein anabolic actions.

Thyroid hormones, especially free triiodothyronine, have been correlated with increased protein turnover in a cold environment. In a preliminary study with rats, cold-exposure augmented both muscle protein synthesis and degradation, and these changes were associated with elevated plasma free triiodothyronine concentrations. (Brown *et al.*, 1984). Enhanced thyroid hormone activity may therefore account for the increased urinary N^{τ}-methylhistidine excretion reported for cold-adapted calves in Chapter II. A linear correlation between *in vitro* rates of muscle and hepatocyte protein synthesis and plasma free triiodothyronine concentration was also demonstrated in sheep injected daily with thyroxine for five weeks (M^{c}Bride and Early, 1989). Therefore, elevated plasma thyroid hormone concentrations may have stimulated muscle protein synthesis in the C90 group of calves so that it was maintained at the same level as the W72 group.

Although there are few reports on the effect of glucagon on protein turnover, increased glucagon in the cold may enhance hepatic extraction of amino acids for gluconeogenesis, probably reducing amino acids available for protein synthesis (Brockman and Laarveld, 1986). Experiments examining the use of amino acids for gluconeogenesis in cold-adapted animals should include determination of hepatic and renal sensitivity and responsiveness to glucagon.

The central nervous system, which regulates the endocrine system, is itself influenced by environmental factors (Young, 1980). When an animal is subjected to a stressful environment, the central nervous system mounts a coordinated response to meet added metabolic requirements, including increased sympatho-adrenal activity. Catecholamines, the effectors of the sympatho-adrenal system, can influence the secretion of and response to several other hormones. Since plasma catecholamine concentrations are elevated in cold-adapted ruminants (Christopherson *et al.*, 1978; Graham *et al.*, 1981), it is probable that elevated catecholamine concentrations coordinate the endocrine alterations observed in the cold-adapted heifers in Chapter III. It appears that ß-adrenergic stimulation of insulin secretion was predominant in the cold-adapted heifers, which is in contrast to reports that an α-adrenergic reduction in insulin secretion was evident in cold-adapted sheep (Sasaki *et al.*, 1982). In addition to influencing secretion, catecholamines could affect insulin binding to its receptors, as Cigolini *et al.* (1986) reported a noradrenaline-induced reduction in insulin receptor affinity in human fat cells. Increased activity of the sympathoadrenal system could also promote the release of thyroid hormones and glucagon, which could in turn act synergistically with catecholamines to enhance mobilization and utilization of energy substates (Young, 1980). Thyroid hormones appear to play a role in increasing heat production in the cold (Christopherson *et al.*, 1978), while stimulation of gluconeogenesis and glycogenolysis by glucagon would elevate glucose concentrations in the cold.

It appears that hormonal alterations observed in the cold-adapted heifers may have helped prevent a fall in muscle and skin protein synthesis in calves in the C90 group. Although increased plasma concentrations of hormones such as insulin and insulin-like growth factor I shift in a direction expected to increase protein synthesis, depressed tissue responsiveness or sensitivity to those hormones cannot be ruled out. In a cold environment, the energy-providing actions of hormones like insulin may take precedence over their protein anabolic effects. A regulatory role for the sympathoadrenal system in the endocrine response to cold-adaptation is indicated.

Methodological Considerations in the Measurement of Protein Synthesis

The continuous infusion method used to measure protein synthesis in Chapter II allows estimation of the fractional synthesis rate of protein in discrete tissues, especially tissues which are turning over slowly, such as muscle and skin (Waterlow *et al.*, 1978). However, it is not as useful for tissues which are turning over rapidly due to recycling of label during the infusion period.

Several assumptions are made when using the method. First, the true precursor pool for protein synthesis is the aminoacyl-tRNA, but due to difficulty in ascertaining its amino acid specific radioactivity, it is represented by the plasma or intracellular free amino acid pool (Waterlow *et al.*, 1978). If the intracellular free amino acid pool is chosen, it is assumed that it rapidly equilibrates with the extracellular or plasma pool (Waterlow *et al.*, 1978). However, due to compartmentation of the free amino acid pool and dilution of the label by amino acids released by proteolysis, the intracellular specific radioactivity will be lower than that of the plasma (Rannels *et al.*, 1982), which was confirmed by the ratio of intracellular to plasma free phenylalanine specific radioactivity in Appendix 3. Larger ratios in omasum and kidney in the C72 group may indicate that the rate of protein degradation is retarded, thus reducing release of unlabeled phenylalanine into the intracellular pool.

The true value of the fractional synthesis rate of protein likely lies between the maximum and minimum estimates, which are calculated using the specific radioactivity of phenylalanine in the intracellular and plasma pools, respectively. Schaefer *et al.* (1986) found that as the concentration of carrier leucine in the infusate was increased from 127 to 380 mM, differences between the specific radioactivity of leucine in the intracellular and plasma pools diminished, and so did differences between maximum and minimum estimates of fractional synthesis rates. In Chapter II, addition of 10 mM phenylalanine to the infusate was inadequate to achieve a similar result, probably because the concentration was too low.

A method which minimizes recycling of label, and thus differences between amino acid specific radioactivity in the plasma and intracellular pools, is the flooding dose technique developed by Garlick *et al.* (1980). All amino acid pools are flooded with a large amount of unlabeled amino acid administered in a single injection along with the label. Intracellular free amino acid specific radioactivity rapidly approaches that of plasma so the choice of precursor pool does not greatly influence results obtained. Tissues are removed shortly after the injection, reducing opportunity for recycling of labeled proteins (Garlick, 1980). Thus, the method is particularly effective for determining the fractional synthesis rate of protein in gastrointestinal tissues.

Recently, Attaix and Arnal (1987) have used a flooding dose of [^{3}H]valine to measure the fractional synthesis rate of protein in gastrointestinal tissues from lambs. Variability for those tissues was much lower than reported in Chapter II, and there was no significant difference between synthesis rates calculated using either intracellular or plasma specific radioactivity as the precursor pool. The flooding dose method may be able to detect changes in protein turnover in gastrointestinal tissues following cold-adaptation. Alterations in protein synthesis are suggested by results demonstrating a faster rate of digesta passage through the gastrointestinal tract of cold-adapted sheep (Westra and Christopherson, 1976) and an extended enteroctyte life span in cold-exposed piglets (Dauncey *et al.*, 1983). Due to the combination of a large mass and a rapid turnover rate, gastrointestinal tissues account for a large proportion of whole-body protein synthesis. Therefore, any effect of cold-adaptation on turnover in these tissues would significantly alter whole-body protein turnover.

Methodological Considerations in the Measurement of Protein Degradation

Millward *et al*. (1981) have suggested that for growing animals, protein turnover be equated to the replacement rate of protein, i.e., the rate of protein degradation. They also suggested that protein degradation is a first order process, occurring at a constant fraction per day, so the absolute rate is dependent on amount of protein present. However, measurement of the rate of protein degradation presents serious methodological problems.

Most measurements of protein degradation *in vivo* are calculated from the difference between fractional rates of protein synthesis and protein growth (Millward *et al*., 1981), resulting in additive errors. Determining protein growth in individual tissues usually requires either comparative slaughter trials, involving the use of several animals, or an assumption that the proportion of body weight contributed by a tissue remains constant over time (see Pell and Bates, 1987). Although urinary excretion of N^{τ}-methylhistidine has been validated for cattle as a measure of muscle protein degradation (Harris and Milne, 1981), the amount of N^{τ}-methylhistidine released from skeletal versus smooth muscle has not been quantitated. Another method for measuring whole-body protein degradation assumes that the inputs to amino acid flux can be partitioned into dietary intake and protein degradation. However, it is difficult to accurately estimate the amount of amino acid actually absorbed by the digestive system into the blood due to uncertainty about the oxidation of amino acids by the gastrointestinal tissues. None of the methods discussed for determining protein degradation are completely satisfactory. Since protein degradation does not always change in the same direction as synthesis, interpretation of the effects of treatment on protein turnover will be incomplete if it is disregarded. Therefore, the development of more reliable methods for determining protein degradation is crucial to understanding protein turnover in its entirety.

Role of RNA in Regulating the Rate of Protein Synthesis

Millward *et al.* (1981) hypothesized that protein synthesis is a first-order process whose rate is determined mainly by the amount of translational apparatus such as the number of ribosomes. This is supported by reports of a linear relationship between RNA content of a tissue (g RNA g^{-1} protein) and the fractional synthesis rate of protein in that tissue (Attaix, 1988; Garlick *et al.*, 1976; Muramatsu and Okumura, 1985). A similar relationship was observed between RNA content (Appendix 7) and the fractional synthesis rates of protein (Chapter II) when data from all tissues are considered (see Figure IV-1).

RNA content and protein synthesis per unit of RNA have been termed the capacity for protein synthesis and the RNA activity, respectively (Millward, 1978). The latter can be modified by homones and nutritional factors, but usually does not vary a great degree either within or between tissues (Millward, 1978). Despite the significant relationship shown in Figure IV-1, when the fractional synthesis rate of protein in a tissue varied with treatment there was no corresponding change in the capacity for protein synthesis or the RNA activity. Determination of RNA content in a tissue can be confounded by protein that is solubilized along with nucleic acids. Muscle and skin, the only tissues where fractional synthesis rates of protein were influenced by treatment, also have a high protein content, which may have masked changes in RNA content. More accurate methods for measuring RNA concentration in tissues with a high protein content is required.

Conclusion:

Results presented in this thesis demonstrate that fractional synthesis rates of protein in muscle and skin are decreased following cold-adaptation in calves on a restricted feed intake. The observed changes in whole-body and tissue protein metabolism were likely mediated by availability of energy and protein as well as the endocrine system. Since a reduction in muscle protein synthesis can affect meat production, further research on the role of protein and energy intake in this response to cold-adaptation is warranted. Additional information on the effect of cold-adaptation on tissue responsiveness or sensitivity to hormones involved in the regulation of protein turnover is also required.

TABLE IV-1: A summary of the fractional synthesis rate (FSR; % day^{-1}) of protein in skin and the contribution of the absolute rate of protein synthesis in skin to whole-body protein synthesis (%) in several animal species.

SPECIES	FSR	% OF WHOLE BODY	REFERENCE
Cattle (236-628 kg BW)	3.9-7.4	14-21	Lobley *et al.*, 1980
Calves (61-72 kg BW)			
W72 and C90	12	9-13	Present study
C72	7	8	"
Lambs (15-24 kg BW)	35	21	Davis *et al.*, 1981
Lambs* (15.9 kg BW)	22	27	Attaix, 1988
Pigs* (10 days old)	26	14	Attaix, 1988
Rats*† (130-140 g BW)			
Fed	64	18¶	Preedy *et al.*, 1983
Fasted 2 days	47		

*: Flooding dose of radiolabeled amino acid used to measure FSR

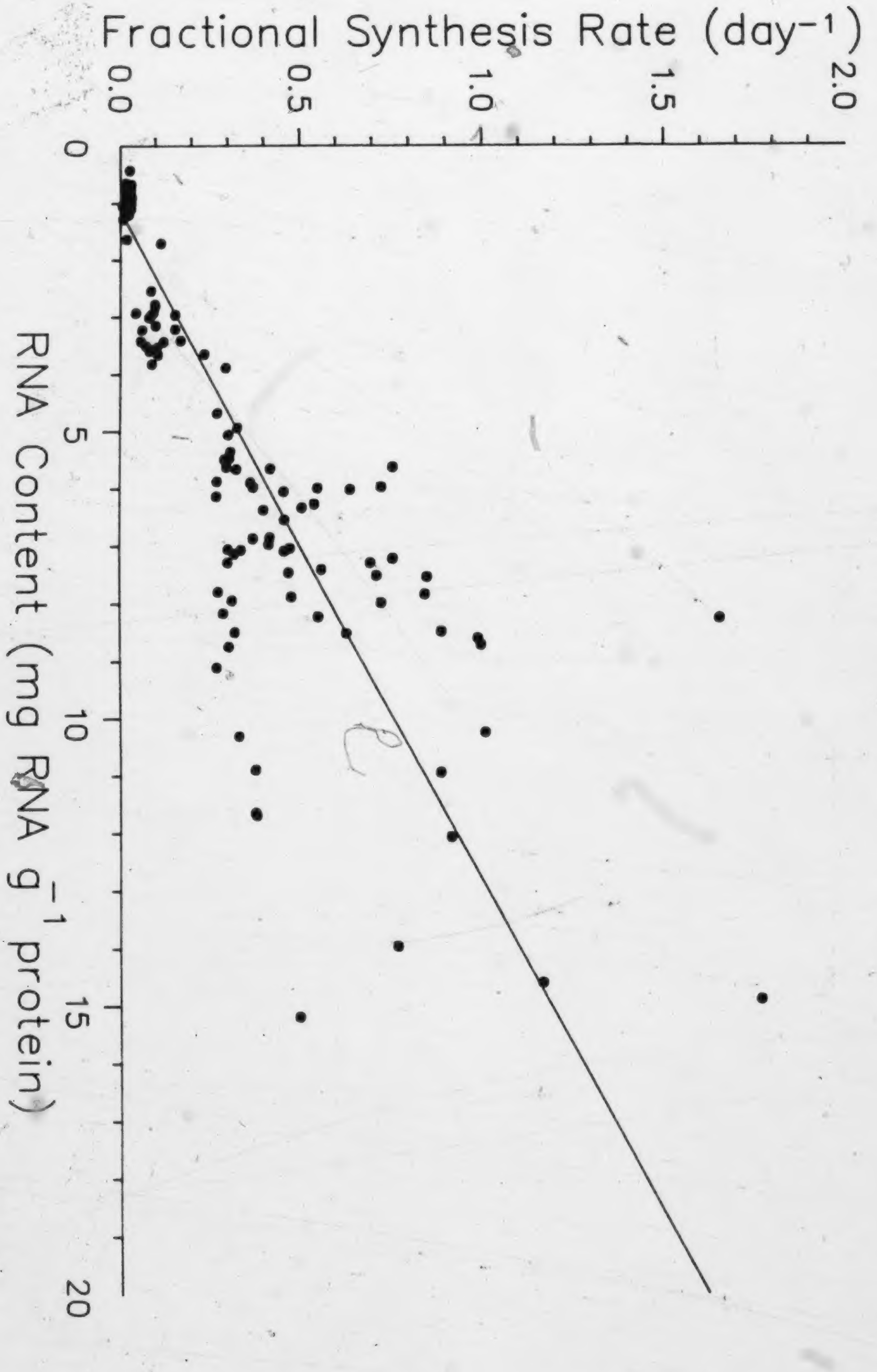
†: Only includes protein soluble in 0.3 M NaOH.

¶: Mean of both treatments.

FIGURE IV-1: The relationsip between RNA content (mg RNA g^{-1} protein) and fractional synthesis rate of protein (% day^{-1}) in calves adapted to two environments. Points are the individual values of ten tissues. The line of best fit is:

$$y = 0.0855x\ (\pm\ 0.0086) - 0.0969\ (\pm\ 0.0559)$$

where y is the fractional synthesis rate of protein and x is RNA content; $n = 109$, $P<0.0001$ for the coefficent, $P>0.05$ for the intercept, $r^2 = 0.47$.


Fractional Synthesis Rate (day⁻¹)
0.0
0.5
1.0
1.5
2.0
RNA Content (mg RNA g⁻¹ protein)
0
5
10
15
20

LITERATURE CITED

Allison, J.B., and R.W. Wannemacher, Jr. 1965. The concept and significance of labile and over-all protein reserves of the body. Am. J. Clin. Nutr. 16: 445-452.

Ames, D.R., and D.R. Brink. 1977. Effect of temperature on lamb performance and protein efficiency ratio. J. Anim. Sci. 44: 136-140.

Ames, D.R., Brink, D.R., and C.L. Willms. 1980. Adjusting protein in feedlot diets during thermal stress. J. Anim. Sci. 50: 1-6.

Aoyagi, Y., Tasaki, I., Okumura, J.-I., and T. Muramatsu. 1988. Effect of low ambient temperature on protein turnover and heat production in chicks. Comp. Biochem. Physiol. 89A: 433-436.

Attaix, D. 1988. Influence de l'âge et du sevrage sur la synthèse protéique chez l'agneau. These de Doctorat d'Etat présenteé à l'Université Blaise Pascal (Clermont II).

Attaix, D., and M. Arnal. 1987. Protein synthesis and growth in the gastrointestinal tract of the young preruminant lamb. Br. J. Nutr. 58: 159-169.

Baracos, V.E., Wilson, E.J., and A.L. Goldberg. 1984. Effects of temperature on protein turnover in isolated rat skeletal muscle. Am. J. Physiol. 246: C125-C130.

Brockman, R.P., and B. Laarveld. 1986. Hormonal regulation of metabolism in ruminants: A review. Livest. Prod. Sci. 14: 313-334.

Brown, J.G., Van Bueren, J., and D.J. Millward. 1984. Differential changes in free and total 3,5,3'-triiodothyronine in cold-exposed rats. Proc. Nutr. Soc. 43: 74A.

Buttery, P.J. 1983. Hormonal control of protein deposition in animals. Proc. Nutr. Soc. 42: 137-148.

Buttery, P.J., and L. Bryan. 1986. Effect of diet and hormones on protein metabolism in muscle. In: Nuclear and Related Techniques in Animal Production and Health. IAEA, Vienna.

Campbell, R.G. 1988. Nutritional constraints to lean tissue accretion in farm animals. Nutr. Res. Rev. 1: 233-253.

Christopherson, R.J., Thompson, J.R., Hammond, V.A., and G.A. Hills. 1978. Effects of thyroid status on plasma adrenaline and noradrenaline concentrations in sheep during acute and chronic cold exposure. Can. J. Physiol. Pharmacol. 56: 490-496.

Cigolini, M., Zancanaro, C., Cavallo, E., Benati, D., Ferrari, S., and O. Bosello. 1986. Long term effect of noradrenaline on insulin binding to human adipose tissue "in vitro". Horm. Metabol. Res. 18: 718-719.

Dauncey, M.J., Ingram, D.L., James, P.S., and M.W. Smith. 1983. Modification by diet and environmental temperature of enterocyte function in piglet intestine. J. Physiol. 341: 441-452.

Davis, S.R., Barry, T.N., and G.A. Hughson. 1981. Protein synthesis in tissues of growing lambs. Br. J. Nutr. 46: 409-419.

Early, R.J., M^c^Bride, B.W., and R.O. Ball. 1988. Phenylalanine metabolism in sheep infused with glucose plus insulin. II. Effects on *in vivo* and *in vitro* protein synthesis and related energy expenditures. Can. J. Anim. Sci. 68: 721-730.

Elsasser, T.H., Rumsey, T.S., and A.C. Hammond. 1989. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. J. Anim. Sci. 67: 128-141.

Fauconneau, B., Arnal, M., and P. Luquet. 1981. *In vivo* protein synthesis in rainbow trout (*Salmo gairdneri* R.): Effect of temperature acclimatization. Reprod. Nutr. Dévelop. 21: 293-301.

Fauconneau, B., and M. Arnal. 1985. *In vivo* protein synthesis in different tissues and the whole body of rainbow trout (*Salmo gairdnerii* R.): Influence of environmental temperature. Comp. Biochem. Physiol. 82A: 179-187.

Fulks, R.M., Li, J.B., and A.L. Goldberg. 1975. Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. J. Biol. Chem. 250: 290-298.

Garlick, P.J. 1980. Assessment of protein metabolism in the intact animal. In: Protein Deposition in Animals, P.J. Buttery, and D.B. Lindsay, eds., pp. 51-67. Butterworths, London.

Garlick, P.J., Burk, T.L., and R.W. Swick. 1976. Protein synthesis and RNA in tissues of the pig. Am. J. Physiol. 230: 1108-1112.

Garlick, P.J., Fern, M., and V.R. Preedy. 1983. The effect of insulin infusion and food intake on muscle protein synthesis in postabsorptive rats. Biochem. J. 210: 669-676.

Garlick, P.J., M^c^Nurlan, M.A., and V.R. Preedy. 1980. A rapid and convenient technique for measuring the rate of protein synthesis in tissues by injection of [^{3}H]phenylalanine. Biochem. J. 192: 719-723.

Goodenough, R.D., Royle, G.T., Nadel, E.R., Wolfe, M.H., and R.R. Wolfe. 1982. Leucine and urea metabolism in acute human cold exposure. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 53: 367-372.

Graham, A.D., Christopherson, R.J., and J.R. Thompson. 1981. Endocrine and metabolic changes in sheep associated with acclimation to constant or intermittent cold exposure. Can. J. Anim. Sci. 61: 81-90.

Harris, P.M., and G.E. Lobley. 1989. Amino acid and energy metabolism in peripheral tissues. Proceedings of the VIIth International Symposium on Ruminant Physiology, August 28-September 1, 1989, Sendai, Japan. Abstract MS3-3, p. 36.

Harris, C.I., and G. Milne. 1981. The urinary excretion of N^{τ}-methylhistidine by cattle: validation as an index of muscle protein breakdown. Br. J. Nutr. 45: 411-422.

Kennedy, P.M., Early, R.J., Christopherson, R.J., and L.P. Milligan. 1986. Nitrogen transformations and duodenal amino acid content in sheep given four forage diets and exposed to warm and cold ambient temperatures. Can. J. Anim. Sci. 66: 951-957.

Lindsay, D.B. 1982. Relationships between amino acid catabolism and protein anabolism in the ruminant. Fed. Proc. 41: 2550-2554.

Lindsay, D.B., Dauncey, M.J., Barker, P.J., and D.L. Ingram. 1988. Protein synthesis and degradation after acclimation to different environmental temperatures and energy intakes. J. Therm. Biol. 13: 79-83.

Lobley, G.E., Milne, V., Lovie, J.M., Reeds, P.J., and K. Pennie. 1980. Whole body and tissue protein synthesis in cattle. Br. J. Nutr. 43: 491-502.

MacRae, J.C., and P.J. Reeds. 1980. Prediction of protein deposition in ruminants. In: Protein Deposition in Ruminants, P.J. Buttery and D.B. Lindsay, eds., pp. 225-249. Butterworths, London.

M^{c}Allister, T. 1987. Effect of temperature on protein turnover in the laboratory rat. M.Sc. Thesis. University of Alberta, Edmonton, Canada.

M^{c}Bride, B.W., and R.J. Early. 1989. Energy expenditure associated with sodium/potassium transport and protein synthesis in skeletal muscle and isolated hepatocytes from hyperthyroid sheep. Br. J. Nutr. 62: 673-682.

M^{c}Kay, D.G. 1974. Utilization of energy substrates by ruminants exposed to cold. Ph.D. Thesis. University of Alberta, Edmonton, Canada.

Millward, D.J. 1978. The regulation of muscle-protein turnover in growth and development. Biochem. Soc. Trans. 6: 494-499.

Millward, D.J., Bates, P.C., and S. Rosochacki. 1981. The extent and nature of protein degradation in the tissues during development. Reprod. Nutr. Dévelop. 21: 265-277.

Millward, D.J., Bates, P.C., deBenoist, B., Brown, J.G., Cox, M., Halliday, D., Odedra, B., and M.J. Rennie. 1983. Protein turnover: The nature of the phenomenon and its physiological regulation. In: Proceedings of the IVth International Symposium on Protein Metabolism and Nutrition, pp. 69-96. INRA Publication No. 16. Clermont-Ferrand, France.

Mount, L.E. 1980. Growth and the thermal environment. In: Growth in Animals, T.L.J. Lawrence, ed., pp. 47-63. Butterworths, London.

Muramatsu, T., and J.-I. Okumura. 1985. Whole-body protein turnover in chicks at early stages of growth. J. Nutr. 115: 483-490.

Pell, J.M., and P.C. Bates. 1987. Collagen and non-collagen protein turnover in skeletal muscle of growth hormone-treated lambs. J. Endocr. 115: R1-R4.

Preedy, V.R., M^{c}Nurlan, M.A., and P.J. Garlick. 1983. Protein synthesis in skin and bone of the young rat. Br. J. Nutr. 49: 517-523.

Prior, R.L., and S.B. Smith. 1983. Role of insulin in regulating amino acid metabolism in normal and alloxan-diabetic cattle. J. Nutr. 113: 1016-1031.

Rannels, D.E., Wartell, S.A., and C.A. Watkins. 1982. The measurement of protein synthesis in biological systems. Life Sci. 30: 1679-1690.

Reeds, P.J., and M.F. Fuller. 1983. Nutrient intake and protein turnover. Proc. Nutr. Soc. 42: 463-471.

Reeds, P.J., Hay, S.M., Glennie, R.T., Mackie, W.S., and P.J. Garlick. 1985. The effect of indomethacin on the stimulation of protein synthesis by insulin in young post-absorptive rats. Biochem. J. 227: 255-261.

Reeds, P.J., and G.E. Lobley. 1980. Protein synthesis: Are there real species differences? Proc. Nutr. Soc. 39: 43-52,

Sasaki, Y., Takahashi, H., Aso, H., Ohneda, A., and T.E.C. Weekes. 1982. Effects of cold exposure on inslin and glucagon secretion in sheep. Endocrin. 111: 2070-2076.

Schaefer, A.L, Davis, S.R., and G.A. Hughson. 1986. Estimation of tissue protein synthesis in sheep during sustained elevation of plasma leucine concentration by intravenous infusion. Br. J. Nutr. 56: 281-288.

Sumner, R., and T.E.C. Weekes. 1983. Effect of insulin on nitrogen excretion in sheep. Proc. Nutr. Soc. 42: 39A.

Waterlow, J.C., Garlick, P.J., and D.J. Millward. 1978. Protein Turnover in Mammalian Tissues and in the Whole Body. Elsevier North Holland, Amsterdam.

Webster, A.J.F., Chlumecky, J., and B.A. Young. 1970. Effects of cold environments on the energy exchanges of young beef cattle. Can. J. Anim. Sci. 50: 89-100.

Webster, A.J.F., Hicks, A.M., and F.L. Hays. 1969. Cold climate and cold temperature induced changes in the heat production and thermal insulation of sheep. Can. J. Physiol. Pharmacol. 47: 553-562.

Weekes, T.E.C., Sasaki, Y., and T. Tsuda. 1983. Enhanced responsiveness to insulin in sheep exposed to cold. Am. J. Physiol. 244: E335-E345.

Westra, R., and R.J. Christopherson. 1976. Effects of cold on digestibility, retention time of digesta, reticulum motility and thyroid hormones in sheep. Can. J. Anim. Sci. 56: 699-708.

Young, B.A. 1975. Temperature-induced changes in metabolism and body weight of cattle (*Bos taurus*). Can. J. Physiol. Pharmacol. 53: 947-953.

Young, V.R. 1980. Hormonal control of protein metabolism, with particular reference to body protein gain. In: Protein Deposition in Animals, P.J. Buttery and D.B. Lindsay, eds., pp.167-191. Butterworths, London.

Yousef, M.K., and R.R.J. Chaffee. 1970. Studies on protein-turnover rates in cold-acclimated rats. Proc. Soc. Exptl. Biol. Med. 133: 801-804.

APPENDIX 1: Nitrogen balalnce in g N day^{-1} of calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
n	4	4	3	
N Intake	234.0^{a} ± 5.2	227.1^{a} ± 9.2	297.1^{b} ± 16.1	0.011
N in Feces	68.3^{a} ± 7.1	65.7^{a} ± 7.1	105.5^{b} ± 8.2	0.012
N in Urine	53.0^{a} ± 9.8	101.4^{b} ± 9.8	$79.1^{a,b}$ ± 11.3	0.024
N Retention	112.7^{a} ±5.1	60.0^{b} ±10.9	112.5^{a} ± 25.8	0.040
Digestible Protein (%)	70.8 ± 2.5	70.9 ± 2.5	64.5 ± 2.9	0.231
Metabolizable Protein (%)	48.1^{a} ± 4.5	26.5^{b} ± 4.5	$37.6^{a,b}$ ± 5.2	0.029

---

^{a,b}: Means in the same row followed by different letters are significantly different ($P<0.05$).

APPENDIX 2: Weights of organs and tissues (g) in calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	544^{a} ± 29	381^{b} ± 41	559^{a} ± 78	0.051
Biceps Femoris	733^{a} ± 43	513^{b} ± 73	767^{a} ± 94	0.059
Skin	4100 ± 216	3532 ± 349	4196 ±460	0.358
Kidney	125 ± 12	144 ± 3	166 ± 24	0.181
Liver	1216 ± 41	1096 ± 76	1105 ± 301	0.810
Heart	331 ± 23	345 ± 21	413 ± 32	0.122
Rumen	1700 ± 148	1546 ± 121	2211 ± 461	0.215
Omasum	337 ± 36	306 ± 54	435 ± 13	0.157
Intestine	2371 ± 160	1997 ± 404	3240 ± 535	0.125

---

^{a,b}: Means in the same row followed by different letters are significantly different (P<0.10).

APPENDIX 3: Ratio (%) of tissue to plasma specific radioactivity of phenylalanine in calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	76.9	68.9	74.8	0.368
	± 4.1	± 4.4	± 2.4	
Biceps Femoris	77.3	71.2	71.3	0.521
	± 1.0	± 6.3	± 2.8	
Skin	48.4	51.1	47.9	0.660
	± 3.4	± 2.3	± 1.1	
Kidney	61.6[a]	72.3[b]	63.9[a,b]	0.045
	± 1.5	± 3.2	± 3.2	
Liver	53.5	60.5	55.0	0.446
	± 3.3	± 4.5	± 4.2	
Heart	69.0	81.8	73.7	0.125
	± 2.7	± 5.3	± 3.4	
Rumen	56.2	56.0	53.5	0.927
	± 6.6	± 4.0	± 2.6	
Rumen Papillae	42.9	45.0	36.5	0.499
	± 1.8	± 4.5	± 8.2	
Omasum	44.8[a]	57.7[b]	47.6[a,b]	0.045
	± 2.8	± 0.2	± 6.0	
Intestine	38.9	42.3	44.7	0.920
	± 8.4	± 10.2	± 11.2	

[a,b]: Means in the same row followed by different letters are significantly different ($P<0.05$).

APPENDIX 4: Table corresponding to Figures II-2A to II-2E presented in Chapter II. Maximum fractional synthesis rate (% synthesized day^{-1}) of protein in tissues from calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	2.52[a]	1.52[b]	2.77[a]	0.020
	± 0.25	± 0.29	± 0.17	
Biceps Femoris	3.05[a]	1.40[b]	2.63[a]	0.001
	± 0.03	± 0.17	± 0.42	
Skin	12.20	6.50	11.47	0.126
	± 1.88	± 0.90	± 3.12	
Kidney	32.37[a]	28.40[b]	31.73[a]	0.015
	± 0.75	± 0.72	± 1.00	
Liver	29.47	32.32	28.53	0.503
	± 0.85	± 2.82	± 2.81	
Heart	9.37	9.37	10.37	0.449
	± 0.60	± 0.25	± 0.87	
Rumen	42.22	47.77	48.30	0.850
	± 7.68	± 4.17	± 13.83	
Rumen Papillae	58.87	63.70	60.00	0.954
	± 8.33	± 9.01	±20.10	
Omasum	70.72	64.22	69.67	0.898
	± 8.50	± 8.39	± 17.13	
Intestine	141.57	101.12	86.77	0.712
	± 62.92	± 28.44	± 39.13	

[a,b]: Means in the same row followed by different letters are significantly different (P<0.05).

APPENDIX 5: Minimum fractional synthesis rate (% synthesized day^{-1}) of protein in tissues from calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	1.90^{a} ± 0.13	1.07^{b} ± 0.23	2.10^{a} ± 0.17	0.010
Biceps Femoris	2.37^{a} ± 0.03	0.97^{b} ± 0.17	1.87^{c} ± 0.23	0.0004
Skin	5.77 ± 0.81	3.30 ± 0.53	5.40 ± 1.45	0.161
Kidney	19.52 ± 0.54	20.20 ± 0.67	19.97 ± 1.62	0.861
Liver	15.42 ± 0.89	18.85 ± 0.87	15.33 ± 1.99	0.113
Heart	6.40 ± 0.24	7.65 ± 0.33	7.60 ± 0.92	0.175
Rumen	21.87 ± 2.24	25.92 ± 3.11	24.57 ± 6.47	0.738
Rumen Papillae	23.95 ± 3.69	28.00 ± 6.26	20.33 ± 7.69	0.675
Omasum	29.22 ± 2.64	35.35 ± 4.31	31.33 ± 7.74	0.645
Intestine	34.47 ± 6.01	33.60 ± 8.51	27.70 ± 3.12	0.776

---

^{a,b,c}: Means in the same row followed by different letters are significantly different ($P<0.05$).

APPENDIX 6: Protein content (mg protein g^{-1} tissue) of tissues from calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	139.0 ± 3.9	134.0 ± 10.8	150.7 ± 5.8	0.379
Biceps Femoris	132.9 ± 2.8	158.4 ± 11.7	134.9 ± 6.9	0.103
Skin	148.6 ± 5.8	136.3 ± 12.4	139.0 ± 12.8	0.678
Kidney	96.6 ± 2.6	121.8 ± 15.3	109.2 ± 7.2	0.261
Liver	139.5 ± 4.9	153.4 ± 10.7	136.8 ± 5.1	0.325
Heart	116.7[a] ± 2.9	133.8[b] ± 2.8	126.5[b] ± 1.3	0.004
Rumen	83.2 ± 9.4	93.4 ± 1.5	77.2 ± 6.5	0.297
Rumen Papillae	91.8 ± 5.0	91.2 ± 6.7	73.4 ± 11.7	0.239
Omasum	73.4 ± 3.6	77.4 ± 4.0	82.5 ± 1.5	0.264
Intestine	85.5 ± 5.3	96.4 ± 7.5	97.0 ± 4.0	0.366

[a,b]: Means in the same row followed by different letters are significantly different ($P<0.05$).

APPENDIX 7: RNA content or capacity for protein synthesis (mg RNA g^{-1} protein) in tissues from calves adapted to two environmental temperatures (Mean ± SEM).

	W72	C72	C90	P VALUE
n	4	4	3	
Longissimus Dorsi	0.89 ± 0.16	1.15 ± 0.17	0.95 ± 0.11	0.489
Biceps Femoris	0.97 ± 0.05	0.93 ± 0.15	0.81 ± 0.09	0.600
Skin	3.14 ± 0.17	3.20 ± 0.15	2.78 ± 0.54	0.583
Kidney	8.65 ± 0.66	8.08 ± 0.38	6.71 ± 0.86	0.160
Liver	4.84 ± 0.37	5.95 ± 0.18	5.00 ± 0.69	0.164
Heart	3.18 ± 0.26	3.33 ± 0.22	3.04 ± 0.20	0.695
Rumen	6.21 ± 0.48	6.88 ± 0.46	6.28 ± 0.48	0.564
Rumen Papillae	7.16 ± 0.38	8.07 ± 0.35	6.92 ± 0.90	0.312
Omasum	8.49 ± 1.88	7.07 ± 0.36	8.10 ± 1.07	0.725
Intestine	11.9 ± 0.9	11.8 ± 1.3	11.7 ± 2.0	0.994

APPENDIX 8: Method for determining fractional synthesis rate of protein in tissues from calves weighing 60-70 kg. Adapted from McAllister (1987).

NOTE: The quantities and concentrations of solutions in this method were used in the analysis of plasma and tissues from Holstein calves weighing 60-70 kg and continuously infused with ^{3}H-Phe at a dose of 40μCi kg^{-1} for 8 hours. Because radioactivity in tissues is lower using this method than with the flooding dose technique (Garlick *et al.*, 1980), every effort is made to avoid unneccessary dilution of samples.

NOTE: The values of *g* force given for centrifugation are maximum values at the bottom of the tube.

1. Tissue homogenization
   a) Using a tissue homogenizer such as a Polytron (1 cm head), grind fresh or thawed tissue samples in a known amount of ice-cold 2% (w/v) perchloric acid (PCA) for approximately 0.5 minute at low speed and 1 minute at high speed. Store homogenate on ice.
   Purpose: To precipitate the tissue proteins, thus separating them from free amino acids.
   Example: 5 g tissue + 15 mL PCA
   -> 10 mL for homogenization
   -> 5 mL for washing homogenizer blades
   b) Centrifuge homogenate at 3100 x *g* for 15 minutes.
   Purpose: To separate precipitated tissue proteins from intracellular free amino acids.
   c) Decant (or pipette) off the supernatant into a storage container. Save the pellet as well. The supernatant is the

intracellular free amino acid fraction and the pellet is the tissue protein fraction.

d) Both fractions can be frozen and stored at this stage.

2. Protein pellets

   a) Wash pellet with ice-cold 2% PCA and shake or vortex vigorously.

      Purpose: To remove any remaining free amino acids from the precipitated proteins.

      Example: Tissue pellet + 10 mL 2% PCA

   b) Centrifuge at 3100 x *g* for 15 minutes.

   c) Discard supernatant into radioactive waste container.

   d) Repeat a), b), and c) two more times (i.e., total of 3 washes).

   e) If analyses on the protein pellet will be performed in addition to this analysis for protein synthesis, it can be dried and/or divided at this point.

   f) Resuspend pellet or portion thereof in 6 N HCl in a Pyrex screw-cap tube.

      Example: 20 mg of pellet + 3 mL of 6 N HCl.

   g) Sparge tubes with nitrogen gas and immediately cap.

   h) Hydrolyze pellets in oven at 110°C for 24 hours; tighten caps after ½ hour in the oven. Solution will turn black.

      Purpose: To hydrolyze protein to free amino acids.

   i) Completely dry hydrolysates using one of the following methods: vacuum centrifuge (Speed-vac concentrator), vacuum oven, or nitrogen gas. If the volume of hydrolysate is large, mix it well and take an aliquot for drying.

Purpose: To remove HCl.

j) Resuspend dried hydrolysate in 0.5 M sodium citrate (pH=6.3).

Example: Dried hydrolysate from f) + 2 mL NaCit.

h) This neutralized solution is used for enzymatic conversion and extraction.

Example: All 2 mL from j) could be used.

3. Intracellular fraction

a) Add saturated tripotassium citrate to the intracellular fraction (homogenate supernatant). The resulting pH should be 5.9 - 7.0. Vortex or shake well. A white precipitate of potassium perchlorate will form.

Purpose: To neutralize the PCA in the intracellular fraction.

Example: 2 mL supernatant + 0.5 mL K_3Cit.

Example: 4 mL supernatant + 1.0 mL K_3Cit.

b) Centrifuge at 3100 x *g* for 15 minutes.

c) Keep supernatant solution for enzymatic conversion and extraction; discard precipitate in radioactive waste.

4. Plasma preparation

a) Add 0.700 mL ice-cold 1.5 N PCA to 2.5 mL plasma. Vortex or shake well. Keep tubes on ice.

Purpose: To precipitate plasma proteins.

b) Centrifuge at 3100 x *g* for 15 minutes.

c) Keep supernatant for neutralization and discard precipitate. (Note: If desired, plasma proteins could be hydrolyzed for analysis according to method given in part 2). Take 2.0 mL of deproteinized plasma and add 0.600 mL of saturated K_3Cit. Vortex or shake well. A white precipitate of potassium perchlorate will form.

Purpose: To neutralize deproteinized plasma.

d) Centrifuge at 3100 x *g* for 15 minutes.

e) Keep supernatant solution for enzymatic conversion and extraction; discard precipitate in radioactive waste.

5. Enzymatic conversion and extraction

Purpose: To convert phenylalanine to ß-phenylethylamine (ß-PEA), thus separating it from other amino acids, such as tyrosine and tyramine, that may have become labeled during infusion.

a) Prepare enzyme suspension (i.e., enzyme will not dissolve) in 0.5 M sodium citrate at pH = 6.3 (suspension may be prepared in advance and stored at -70°C):

L-tyrosine decarboxylase:	2 Units/mL	(Sigma T4379)
Pyridoxyl phosphate:	1 mg/mL	(Sigma P9255)

b) Add 0.5 mL of enzyme suspension to 1.0 mL neutralized intracellular fraction or neutralized deproteinized plasma. Add 0.6 mL of enzyme to the entire 2 mL of reconstituted protein hydrolysate. Stopper and shake.

c) In order to determine the concentration of any background ß-PEA present in the intracellular fraction, substitute 0.5 mL of 0.5 M sodium citrate (pH = 6.3) for the enzyme suspension; the rest of the analysis will be the same as samples receiving enzyme.

d) Incubate in a 50°C waterbath overnight.

e) The next day add 0.5 mL of 3 N NaOH and 5.0 mL chloroform:n-heptane (1:3 v/v) to incubated solution. Shake well. (Note: for extraction procedure, shaking about 60 times works better than vortexing).

Purpose: The α-amino acids become basic, but the ß-PEA becomes neutral (hydrophobic) and can thus be extracted from the basic aqueous layer into the neutral organic layer.

f) Centrifuge at 570 x *g* for 5 minutes.

g) Pipette off the top layer. Make sure not to touch the bottom enzyme layer with the pipette tip or the upper fraction will be contaminated with other amino acids. Add 2.5 mL chloroform and 2.0 mL 0.1 M H_2SO_4. Shake well.

h) Centrifuge at 570 x *g* for 5 minutes.

i) Save the top acidic aqueous layer (approximately 2 mL). This is the final extract which contains ß-PEA; it will be used for HPLC analysis and radioactivity counting.

6. HPLC Analysis

Combine the final extract with ethanolamine, saturated potassium borate solution, and distilled deionized water according to the method given in Appendix 7. Vortex and determine ß-PEA concentration in nmol/mL (μM). (Note: It is best to do HPLC analysis before counting in case repeats are necessary).

7. Radioactivity counting

   a) In a 20 mL scintillation vial, add 12 mL of scintillation fluid to the final extract. Count as much of the final extract as possible. Using 0.1 M H_2SO_4, make sure that the scintillation fluid is miscible with the volume of extract that will be used.

   b) Shake very well, dark adapt, and count. Allow 10,000 counts to accumulate to reduce counting error.

   c) Correct cpm for background and convert net cpm to dpm. Express radioactivity in dpm mL^{-1}.

8. Specific radioactivity of phenylalanine

   a) This calculation assumes that the tritium atom on ^{3}H-phenylalanine has no effect on its conversion to ß-PEA, therefore the specific radioactivity of phenylalanine will be equal to that of ß-PEA.

   b) The specific radioactivity (SRA) of ß-PEA is calculated by dividing the radioactivity of the final extract by the concentration of ß-PEA in the final extract:

$$\text{SRA of ß-PEA} = (\text{dpm mL}^{-1}) \div (\text{nmol mL}^{-1})$$
$$= \text{dpm nmol}^{-1}$$

References:

Garlick, P.J., M^{c}Nurlan, M.A., and Preedy, V.R. 1980. A rapid and convenient technique for measuring the rate of protein synthesis in in tissues by injection of [^{3}H]-phenylalanine. Biochem. J., 192:719-723.

McAllister, T. 1987. The effect of environmental temperature on protein turnover in the laboratory rat. M.Sc. Thesis, University of Alberta.

APPENDIX 9: Detailed experimental methods for Chapter II.

Animals and Management:

Thirteen bull calves 7 days of age were obtained from the University of Alberta Dairy Unit. Calves were housed in individual pens and fed milk diluted with water 2:1 (milk:water, v/v) and a calf starter ration (Co-op Complete Calf Ration I, minimum 15% C.P.; Federated Co-operatives, LTD., Saskatoon, Canada) *ad libitum* until 21 days of age. Amount of unconsumed calf starter was recorded each morning. Starting at 21 days of age, calf starter was substituted with a pelleted ration (Ingredients on a dry matter basis: 20.8% dehydrated alfalfa, 56.59% barley grain, 10.2% dehydrated beet pulp, 6.96% soybean meal, 1.74% molasses, 0.54% $CaPO_4$, 0.56% $CaCO_3$, 0.56% NaCl, 0.56% vitamin ADE premix, 1.68% Permapel pelleting agent, 16.9% crude protein, 16.3 MJ GE kg^{-1}DM) in the following proportions: 2:1, 1:1, and 1:2 (calf starter:pelleted ration, w/w). Each mixture was fed for 1 day. At 28 days of age, calves were weaned by further diluting milk with water 1:1 (milk: water, v/v) for 2 days, then 1:2 (milk:water, v/v) for 2 days.

Two calves died before reaching 35 days of age. At 35 days of age, the 11 remaining calves were moved to individual metabolic crates in temperature-controlled chambers and were randomly assigned to one of three treatment groups: 1) warm environment (20°C), 72 g feed $kg^{-0.75}$BW d^{-1}, 2) cold environment (-5°C), 72 g feed $kg^{-0.75}$BW d^{-1}, or 3) cold environment (-5°C), 90 g feed $kg^{-0.75}$BW d^{-1}. The lower level of feed intake was designed to meet NRC requirements at thermoneutrality (NRC, 1978), while the higher intake of the C90 group was designed to approximate the usual response in *ad libitum* intake during adaptation to a cold environment. Calves were allowed to adapt to treatments for 21 days, during which time the pelleted ration was fed every 2 hours from an automatic feeder. The weight of any unconsumed feed was recorded each morning. Each week, calves were weighed, representative feed samples were taken, and the daily amount of feed was adjusted accordingly. Temperatures in the metabolic chambers were measured with three thermocouples per room and recorded every minute by a Honeywell Electronik 15 Stripchart Multipoint Recorder (Honeywell, Inc., Philadelphia, USA).

Nitrogen and Energy Balance:

Starting at 49 days of age, total collection of urine and feces was undertaken for 7 d at the same time each day. Urine was collected in a large plastic tub under the metabolic crate; 100 mL of 6 N HCl was added to the tub each morning to inhibit bacterial growth. The volume of urine and the weight of feces excreted by each calf were determined each day. Each sampling day, urine was well mixed and a 20 mL aliquot was taken to determine N^{τ}-methylhistidine concentration. In addition, a 10% representative sample of both urine and feces was taken each day. Representative samples for each calf were pooled for the week and frozen at -30°C until analyzed. Pooled fecal samples were weighed and oven dried at 60°C for 5 days, at which time they were ground through a 1 mm mesh in a feed mill (Thomas-Wiley Laboratory Mill Model 4; Arthur H. Thomas Co., Philadelphia, USA). Weekly feed samples for each animal were also pooled and ground in the feed mill. Dry matter content of ground fecal and feed samples was determined after drying at 110°C to constant weight. Crude protein and energy content of urine, fecal, and feed samples was determined by the Kjeldahl method (AOAC, 1984) and bomb calorimetry, respectively.

In the middle of the week of nitrogen and energy balance, oxygen exchange of the calf during an 8 hour period was measured by indirect calorimetry in an open-circuit system as described by Young *et al.* (1975). Room air was sampled as the incoming air and air drawn through a hood enclosing the calf's head was sampled as the outgoing air. Prior to measurement of oxygen consumption, calves were adapted to wearing the hood. A shelf inside the hood held containers of water and feed. During the measurement of oxygen consumption, water was available in the hood and feed was given every 2 hours through a port in the hood. The flow rate of outgoing air (V_2; mL s^{-1}) was monitored with a flowmeter (Rotameter Model 10A-3555A; Fisher and Porter Ltd., Warminster, USA). Oxygen concentration in the incoming (O_1) and outgoing (O_2) air was continuously monitored by a dual-channel paramagnetic analyzer (Servomex Model OA184; Taylor Servomex, Crowborough, U.K.). Prior to reaching the oxygen analyzer, outgoing air was dried through a column of anhydrous $CaSO_4$ (Drierite; W.A. Hammond Drierite Co., Xenia, USA). The baseline oxygen concentration of incoming air was assumed to be 20.95%. The ventilation rate of the hood was adjusted for each calf such that the decrement in oxygen concentration in the outgoing air was approximately 0.5%. Barometric and in-line air pressures, as well as outgoing air temperature and relative humidity were used to adjust observed flow rate to a dry equivalent flow at a standard temperature and pressure (0°C and

760 mm Hg). The whole respiratory gas exchange system was calibrated by the iron-burner procedure of Young et al. (1984). Metabolic rate in watts was calculated using the equation of M^cLean (1972), i.e.,

$$\text{Joules/second} = 20.47\ V_2\ (O_1 - O_2)$$

In addition, rectal and room temperatures were monitored with copper-constantan thermocouples calibrated against an immersible Hg-in-glass thermometer to within ± 0.1°C. Electronic output from the oxygen analyzer and thermocouples was automatically scanned every second by a data acquisiton system (Datataker model DT100 Data Logger; Data Electronics, Box Hill, Australia) and averaged over a preset time. Processed data was stored in the Datataker and was also relayed to a microcomputer (Tandy 1000 Personal Computer; Intertan Inc., Barrie, Canada). This connection allowed the data to be transferred to floppy diskettes for further data processing. Electronic output was monitored concurrently by a millivolt chart recorder (Fisher Recordall Series 5000; Fisher Scientific Co., Fair Lawn, USA).

Measurement of Protein Synthesis:

At 55 days of age, percutaneous catheters were inserted into both of each calf's jugular veins. The right jugular catheter was the infusion line and the left jugular catheter was the sampling line. In order to maximize circulation of the labeled [^{3}H]-phenylalanine prior to sampling, catheters of differing lengths were used. The right jugular catheter was approximately 0.5 m long and its tip ended in the right ventricle; this was achieved by threading catheter tubing containing a small bubble into the jugular vein until the bubble pulsed with the heartbeat. The left jugular catheter was approximatley 0.1 m long and its tip ended in the jugular vein.

At 56 days of age, each calf was given a continuous infusion of L-[ring-2,6-^{3}H]-phenylalanine (59 Ci mmol^{-1}, 1.0 Ci mL^{-1}; Amersham Corp., Oakville, Canada) in sterile physiological saline (10 mmol phenylalanine L^{-1}; 40 μCi kg^{-1}BW) at a rate of 30 mL hour^{-1} for 8 hours. The infusate was made on the day prior to the infusion using a gravimetric method and assuming that all solutions had a density of 1 g mL^{-1}. The total amount of radioactivity required in the infusate was determined from the calf's body weight; a tared sterile syringe was filled with the necessary weight of labeled ^{3}H-phenylalanine and set aside until required. Next, 0.40474 g L-phenylalanine (Sigma Chemical Co., St. Louis, USA) and 60 mL of sterile saline were added to a tared sterile Erlenmyer flask. The labeled ^{3}H-phenylalanine was injected into the flask and the syringe rinsed several times. Additional saline was added to the flask to a weight of 245.00 g. The infusate was stored overnight at 4°C. To sterilize the infusion line (Argyle Medicut Sentinel Line tubing; Ingram and Bell, LTD., Don Mills, Canada), a solution of ethanol (70 %, v/v; Fisher Scientific Co., Fair Lawn, USA) was pumped through with a peristaltic pump (Pharmacia Peristaltic Pump P-3 with 2.1 mm i.d. silicon tubing; Pharmacia Fine Chemicals, Uppsala, Sweden) followed by sterile saline. The infusion line was threaded through a long length of plastic tubing approximately 2 cm in diameter, and this plastic tubing was in turn passed through a port in the chamber in which the animal was housed. Immediately prior to the infusion, the peristaltic pump was calibrated by measuring the amount of saline pumped through the infusion line in 20 minutes. This process also served to determine the lag time until the infusate reached the infusion catheter.

After the infusion line was connected to the infusion catheter, sterile saline was pumped into the calf. When the infusion took place in the cold chamber, the temperature in the chamber was increased to 0°C to prevent the infusate from freezing in the line. A 10 mL blood sample was taken and immediately placed on ice in a test tube containing 140 USP units of heparin sodium (Allen and Hanburys, Toronto, Canada). At the beginning of the infusion, the Erlenmyer flask containing the infusate was set on a balance. Its weight was checked at hourly intervals to determine the actual flow rate during the infusion. The timing for the infusion started after the aforementioned lag time had elapsed. During the 8 hour infusion, the calf was watched carefully to prevent disconnection of the infusion line. Additional blood samples were taken every 20 minutes and stored on ice in heparinized test tubes. Within 2 hours, blood samples were centrifuged at 2280 x *g* for 15 minutes. Plasma was removed and stored at -70°C until analyzed.

At the conclusion of the 8 hour infusion, the calf was injected with thiopentone sodium (Intraval sodium, 1 mL 2.72 kg^{-1}BW; M.T.C. Pharmaceuticals, Mississauga, Canada) and bled by decapitation. Tissue samples of the rumen, rumen papilla, omasum, intestine (duodenum), kidney, liver, heart, longissimus dorsi, biceps femoris, and skin (perianal) were immediately removed, rinsed with cold sterile saline if necessary, and placed in small aluminum pans on ice. The tissue samples were weighed, minced with scissors, and homogenized in 10 mL ice-cold 2% (v/v) perchloric acid (Fisher Scientific Co., Fair Lawn, USA) with a

Polytron homogenizer (Brinkman Instruments, Rexdale, Canada) to precipitate tissue proteins. The homogenizer blades were rinsed with an additional 5 mL of ice-cold 2% PCA for all tissues except the skin, which was rinsed with an additional 10 mL of ice-cold 2% PCA. Samples were removed and homogenized as quickly as possible. The homogenate was centrifuged at 2280 x *g* for 15 minutes at 4°C to separate the protein pellet from the acid supernatant. The supernatant was decanted from the pellet and both were frozen at -50°C until further analysis. Samples of the infusate were also taken and frozen at -50°C. The homogenate pellet was assumed to represent the tissue protein fraction and the homogenate supernatant was assumed to represent the tissue intracellular fraction.

The protein pellets were used for three analyses: 1) rate of protein synthesis, 2) tissue protein content, and 3) tissue RNA content. Measurement of protein synthesis required determination of the specific radioactivity of phenylalanine bound in tissue proteins. An enzymatic decarboxylation of phenylalanine to ß-phenylethylamine followed by organic extraction of ß-phenylethylamine was necessary to separate the labeled phenylalanine from any of its labeled products such as tyrosine and tyramine. The method used was the modification of an original method of Suzuki and Yagi (1976) outlined by McAllister (1987). This method, which was also used to determine the specific radioactivity of free phenylalanine in the intracellular fraction and plasma, can be found in Appendix 5.

Prior to any analysis, the protein pellet was washed twice with 10 mL ice-cold 2% perchloric acid to remove any free amino acids. After each wash the pellet was centrifuged at 3100 x *g* for 15 minutes and the supernatant discarded. The pellets were weighed before and after lyophilization. A small trial with pure bovine serum albumin (Sigma Chemical Co., St. Louis, USA) showed that the perchlorate ion contributed approximately 17% to the weight of the freeze-dried pellet.

HPLC Analyses:

The concentration of ß-phenylethylamine in the final extracts was determined using high performance liquid chromatography (HPLC) according to the method outlined by M^{c}Allister (1987). Ethanolamine (Fisher Scientific Co., Fair Lawn, USA) was used as an internal standard for all samples. Standard solutions of ethanolamine and ß-phenylethylamine (Sigma Chemical Co., St. Louis, USA) were made up daily in HPLC grade water (Fisher Scientific Co., Fair Lawn, USA). Saturated potassium borate (BDH Chemicals Ltd., Poole, U.K.) made up in HPLC grade water was used to raise the pH of HPLC samples.

Protein pellet final extracts were prepared for HPLC analysis by combining 10 μL of the final extract with 200 μL of ethanolamine, 200 μL of saturated potassium borate, and 800 μL of HPLC water in 2.0 mL vials. The HPLC system consisted of a Varian model 8085 autosampler (Varian, Walnut Creek, USA), a Varian model 5000 high performance liquid chromatograph (Varian, Walnut Creek, USA) and a Fluoriochrom detector (Varian, Walnut Creek, USA). ß-Phenylethylamine was derivatized with an *o*-phthaldialdehyde reagent (Jones and Gilligan, 1983) composed of 0.50 g *o*-phthaldialdehyde (Sigma Chemical Co., St. Louis, USA), 12.5 mL of methanol (Fisher Scientific Co., Fair Lawn, USA), 112 mL of 0.04 M sodium borate buffer at pH 9.5 (Fisher Scientific Co., Fair Lawn, USA), 0.50 mL of 2-mercaptoethanol (Sigma Chemical Co., St. Louis, USA), and 4.0 mL of Brij 35 (Fisher Scientific Co., Fair Lawn, USA). Samples were mixed 1:1 in the sample vial with the fluoraldehyde reagent prior to injection by a modified Technicon autoanalyzer proportionating pump (Tarrytown, USA) with a stainless steel mixing "T" connector. The 12 sec delay between the "T" connector and the column allowed adequate time for derivatization. The derivatized sample was injected onto a Supelcosil 3 μm LC-18 reverse phase column (4.6 mm I.D. x 75 mm; Supelco Inc., Bellefonte, USA) by a Valco autoinjector valve (Valco Instruments Co., Houston, USA) fitted with a 25 μL loop. A guard column 4.6 mm I.D. x 50 mm (Supelco Inc., Bellefonte, USA) packed with 20-40 μm particles (Supelco Pelliguard LC-18; Supelco Inc., Bellefonte, USA) protected the main column. Two solvents pumped at a rate of 1.5 mL minute^{-1} formed the following gradient: 40% B at time 0 min, 90% B at 4 min and 40% B at 4.5 min. Solvent A was 0.1 M sodium acetate:methanol:tetrahydrofuran (Fisher Scientific Co., Fair Lawn, USA) and solvent B was methanol. Wavelengths for excitation and emission in the fluorometer were 340 nm and 450 nm, respectively. Peaks were concurrently recorded on a Fisher millivolt chart recorder (Fisher Scientific Co., Fair Lawn, USA) and integrated by a Hewlett-Packard 1000E series computer data system via a Hewlett-Packard 18652A A/D converter (Hewlett-Packard Co., Cupertino, USA).

Final extracts of the intracellular fraction were prepared for HPLC analysis by combining 40 μL of the final extract with 20 μL of ethanolamine and 40 μL of saturated potassium borate in 100 μL vials. Plasma final extracts were prepared for HPLC analysis by combining 20 μL of the final extract with 20 μL of ethanolamine, 20 μL of saturated potassium borate, and 40μL HPLC grade water in 100 μL vials. The HPLC system consisted of a Varian Vista 5500 high performance liquid chromatograph, a Varian 9090 Autosampler, and a Varian 2070 Spectrofluorometer (Varian, Walnut Creek, USA). The remainder of the analysis was identical to the one for protein hydrolysates.

Concentrations of free phenylalanine in the intracellular fraction and of bound phenylalanine in the protein fraction were determined by HPLC analysis in the heart, longissimus dorsi, and biceps femoris. A 1.2 mL aliquot of the tissue homogenate supernatant was placed on ice followed by centrifugation at 2280 x *g* for 15 minutes. Next, 0.500 mL of the supernatant was mixed on ice with 0.500 mL saturated potassium borate, 0.100 mL ethanolamine, and 0.200 mL HPLC grade water. A precipitate of potassium perchlorate formed and samples were centrifuged at 2280 x *g* for 15 minutes. The supernatant mixture was placed in 2.0 mL vials and analyzed using the HPLC system described for the analysis of ß-phenylethylamine in protein pellet extracts. Solvents A and B described above were used to form the following gradient: 38% B at 0 min, 50% B at 6 min, 80% B at 6.1 min, 80% B at 6.9 min, 38% B at 7 min. For the determination of protein-bound phenylalanine, about 20 mg of the freeze-dried protein pellet was hydrolyzed in 3 mL 6 N HCl in screw-cap tubes sparged with nitrogen. Samples were placed in a 110°C oven for 24 hours; screw caps were tightened after 0.5 hour. After addition of 0.200 mL 25 mM ethanolamine directly to the hydrolysate, samples were mixed well and centrifuged at 2280 x *g* for 15 min. A 2 μL aliquot of the hydrolysate/ethanolamine mixture was combined with 100 μL saturated potassium borate and 1.30 mL HPLC grade water in 2.0 mL vials. Samples were analyzed using the HPLC system described above for the determination of free phenylalanine in the supernatants.

A third HPLC analysis for N^{τ}-methylhistidine was performed on daily urine samples using modifications of the methods of Nakamura and Pisano (1976) and Wassner, Schlitzer, and Li (1980). To 200 μL of urine or standard (500 μM N^{τ}-methylhistidine; Sigma Chemical Co., St. Louis, USA) 200 μL of 500 μM histidinol (Sigma Chemical Co., St. Louis, USA) were added as an internal standard. Samples were deproteinized with 200 μL 3.0 M perchloric acid and centrifuged at 2280 x *g* for 15 minutes. A 100 μL aliquot of the deproteinized sample was combined with 400 μL HPLC grade water, 40 μL 1.5 M NaOH, and 400 μL of 0.2 M sodium borate at pH 9.0 in a screw-cap tube. While the sample was being vortexed, 250 μL fluorescamine solution (160 mg fluorescamine 100 mL^{-1} acetonitrile; Sigma Chemical Co., St. Louis, USA, and Fisher Scientific Co., Fair Lawn, USA, respectively) were added. Samples were allowed to sit for a few seconds to consume excess fluroescamine, followed by addition of 400 μL of 2.0 M HCl. The vials were mixed, capped, and incubated for 45 minutes at 90°C in a water bath. Samples were extraced twice with 1.5 mL of diethyl ether (Fisher Scientific Co., Fair Lawn, USA) to remove a contaminant (possibly from the feces) that coeluted with N^{τ}-methylhistidine. After incubation, samples were analyzed on a HPLC system consisting of a Varian Vista 5500 high performance liquid chromatograph, a Varian 9090 Autosampler, and a Varian 2070 Spectrofluorometer (Varian, Walnut Creek, USA). The sample was injected onto a Supelcosil 3 μm LC-18 reverse phase column (4.6 mm I.D. x 150 mm; Supelco Inc., Bellefonte, USA). A guard column 4.6 mm I.D. x 50 mm

(Supelco Inc., Bellefonte, USA) packed with 20-40 μm particles (Supelco Pelliguard LC-18; Supelco Inc., Bellefonte, USA) protected the main column. Two solvents pumped at a rate of 1.1 mL minute^{-1} formed the following gradient: 25% B at time 0 minutes, 25% B at 1.0 minutes, 35% B at 3.0 minutes, 45% B at time 9.0 minutes, 60% B at time 9.5 minutes, and 25% B at time 10.0 minutes. Buffer A was 8 mM ammonium acetate at pH 5.0 (Fisher Scientific Co., Fair Lawn, USA) and buffer B acetonitrile.

Tissue protein content was determined using the Hartree (1972) method. Approximately 15 mg of the freeze-dried pellet were solubilized in 10 mL 1.0 N NaOH during an overnight incubation in a 50°C waterbath. The next day, 50 μL of this solution was diluted to 1.0 mL with deionized water and treated with 0.9 mL of Reagent A (2 g sodium-potassium tartrate and 100 g Na_2CO_3 dissolved in 500 mL 1 N NaOH and diluted to 1 L with water). A blank tube and standard tubes containing 0 to 100 μg bovine serum albumin (Sigma Chemical Co., St. Louis, USA) were treated in the same way. The tubes were warmed for 10 minutes in a water bath maintained at 50°C, cooled to room temperature (21-25 °C; approximately 20 minutes) and treated with 0.1 mL of Reagent B (2 g potassium sodium tartrate and 1 g $CuSO_4 \cdot 5H_2O$ dissolved in 90 mL water followed by addition of 10 mL of 1 N NaOH). After 15 minutes, 3 mL of Reagent C (1 vol Folin-Ciocalteu reagent diluted with 15 vol of water; prepared daily; between 0.15 N and 0.18 N when titrated to pH 10 with 1 N NaOH) were rapidly forced into the tubes and then samples were warmed at 50°C for 10 minutes. The absorbance of the samples was read at 650 nm after cooling to room temperature (21-25 °C; approximately 20 min) in a Spectronic 21 spectrophotometer (Milton Roy Co., Rochester, USA).

RNA content of freeze-dried pellets was determined according a modification of the method of Munro and Fleck (1969). Approximately 25 mg of the freeze-dried pellet were solubilized in 4 mL 0.3 N potassium hydroxide during a 1 hour incubation in a 37°C waterbath. Following incubation, samples were cooled on ice then combined with 2.5 mL of 1.2 N perchloric acid to reprecipitate protein and DNA. After 10 minutes on ice to ensure completion of precipitation, samples were centrifuged at 2280 x *g* for 15 minutes and the supernatant filtered through Whatman #4 filter paper (Whatman International LTD., Maidstone, U.K.) into volumetric flasks. Precipitates were washed twice with 5 mL 0.2 N perchloric acid and centrifuged; washings were also filtered into the volumetric flask. Additional perchloric acid was added so that the RNA solution would be 0.1 N perchloric acid when brought up to volume. A standard solution of calf liver RNA (Sigma Chemical Co., St. Louis, USA) was used to make up standards with a final concentration of 0, 2.5, 5.0, 12.5, and 25 μg RNA mL^{-1}. Standards were processed by the same procedure as the samples. Absorbance of standards and samples was read at a wavelength of 260 nm in a dual-beam ultraviolet spectrophotometer (Unicam SP1800 Ultraviolet Spectrophotometer; Pye-Unicam LTD., Cambridge, U.K.). Peptides that became acid-soluble would also absorb light at this wavelength, causing sample absorbance readings to be overestimated. The concentration of protein in the RNA solution was measured using the Bradford method (Bradford, 1976) as modified by Read and Northcote (1981) with bovine serum albumin as the standard. The absorbance of the RNA solution was corrected by subtracting 0.001 O.D. units for each 1 μg protein found per mL of solution.

LITERATURE CITED

Association of Organic and Agricultural Chemists. 1984. Official Methods of Analysis. 14th edition. Method 2.057. A.O.A.C., Arlington, USA.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Hartree, E.F. 1972. Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal. Biochem. 48: 422-427.

Jones, B.N., and J.P. Gilligan. 1983. *o*-Phthaldialdehyde precolumn derivatization and reversed-phase high-performance liquid chromatography of polypeptide hydrolysates and physiological fluids. J. Chromatog. 266: 471-482.

McAllister, T. 1987. Effect of temperature on protein turnover in the laboratory rat. M.Sc. Thesis. University of Alberta, Edmonton, Canada.

McLean, J.A. 1972. On the calculation of heat production from open-circuit calorimetric measurements. Br. J. Nutr. 27: 597-600.

Munro, H.N., and A. Fleck. 1969. Analysis for nitrogenous constituents. In Mammalian Protein Metabolism, vol. III, p. 424-525. H.N. Munro, ed. Academic Press, New York.

Nakamura, H., and J.J. Pisano. 1976. Fluorescamine derivatives of histidine, histamine, and certain related imidazoles: unique fluorescence after heating in acid. Arch. Biochem. Biophys. 177: 334-335.

National Research Council. 1978. Nutrient Requirements of Dairy Cattle, 5th edition. National Academy of Sciences, Washington, D.C.

Read, S.M., and D.H. Northcote. 1981. Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Anal. Biochem. 116: 53-64.

Suzuki, O., and K. Yagi. 1976. A fluorimetric assay for ß-phenylethylamine in rat brain. Anal. Biochem. 75: 192-200.

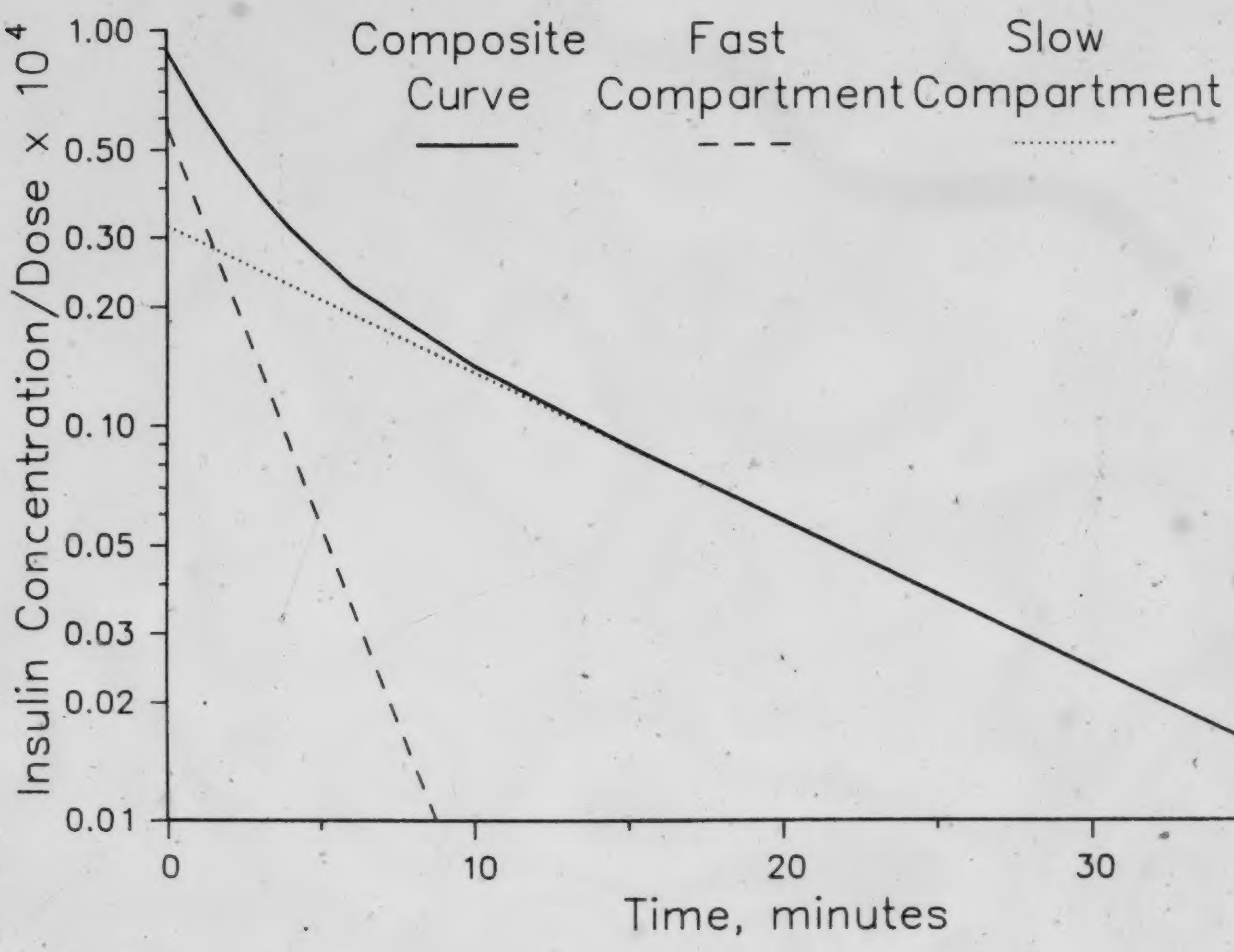
Wassner, S.J., Schlitzer, J.R., and J.B. Li. 1980. A rapid, sensitive method for the determination of 3-methylhistidine levels in urine and plasma using high-pressure liquid chromatography. Anal. Biochem. 104: 284-289.

Young, B.A., Fenton, T.W., and J.A. M^{c}Lean. 1984. Calibration methods in respiratory calorimetry. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 56: 1120-1125.

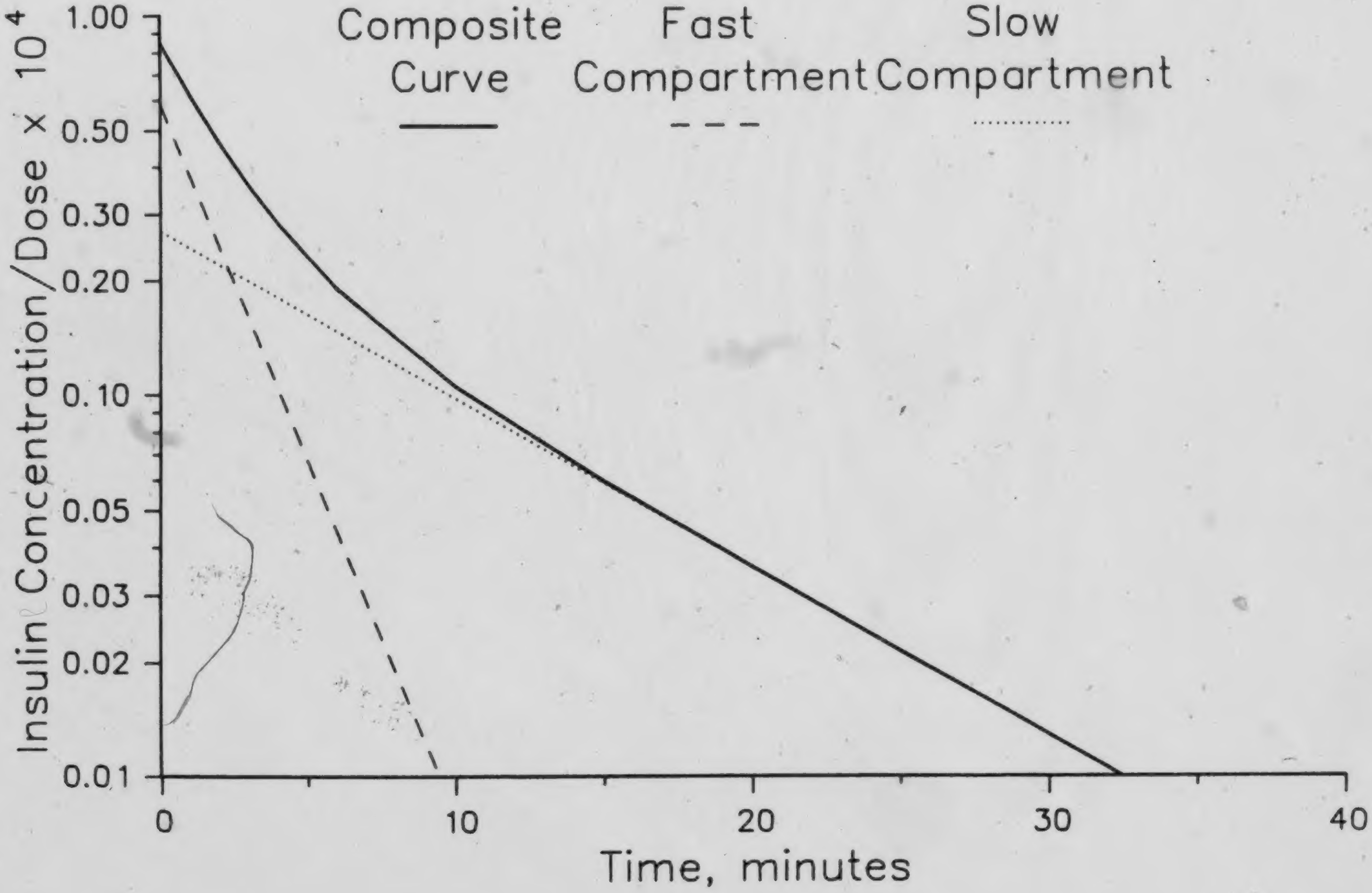
Young, B.A., Kerrigan, B., and R.J. Christopherson. 1975. A versatile respiratory pattern analyzer for studies of energy metabolism of livestock. Can. J. Anim. Sci. 55: 17-22.

APPENDIX 10: Figures corresponding to kinetic parameters for insulin and growth hormone in Tables III-2 and III-3, respectively, presented in Chapter III.

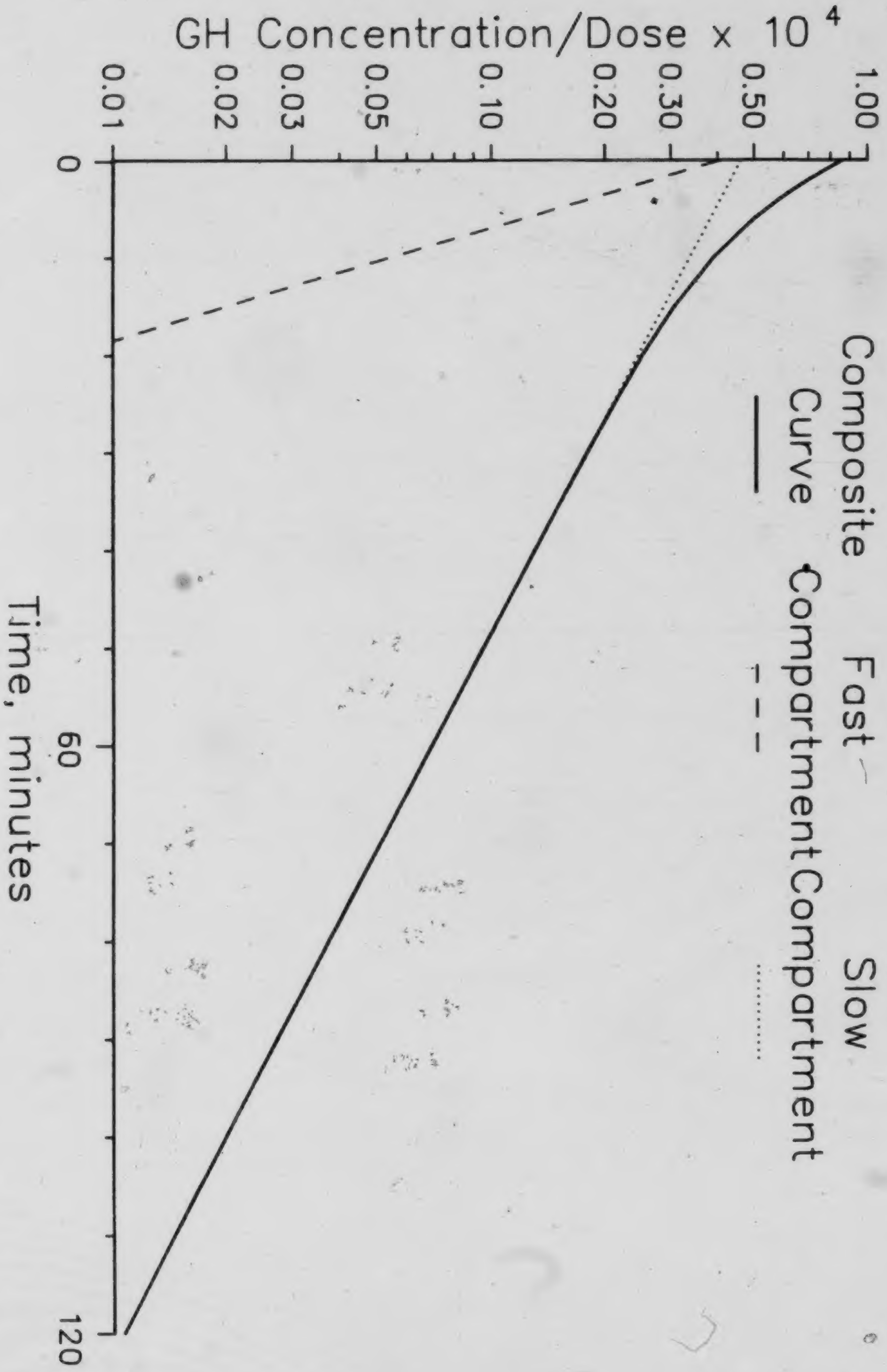
APPENDIX 10A: Mean insulin kinetic curve for heifers adapted to a warm environment.


Insulin Concentration/Dose x 10^4
1.00
0.50
0.30
0.20
0.10
0.05
0.03
0.02
0.01
Composite Curve
Fast Compartment
Slow Compartment
0
10
20
30
40
Time, minutes

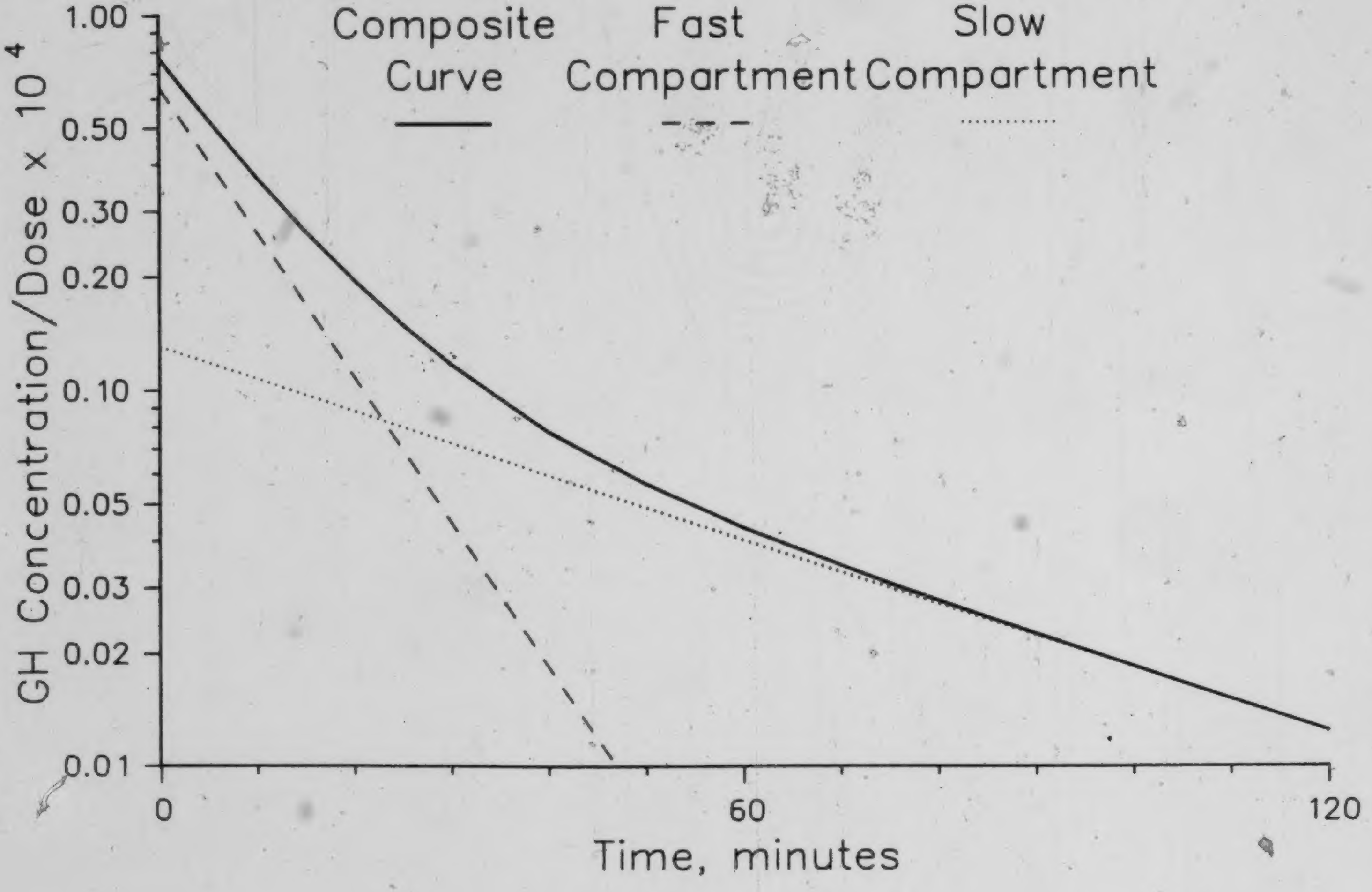
APPENDIX 10B: Mean insulin kinetic curve for heifers adapted to a cold environment.


Composite Curve
Fast Compartment
Slow Compartment
Insulin Concentration/Dose x 10^4
1.00
0.50
0.30
0.20
0.10
0.05
0.03
0.02
0.01
0
10
20
30
40
Time, minutes

APPENDIX 10C: Mean growth hormone kinetic curve for heifers adapted to a warm environment.


GH Concentration/Dose x 10 ^{4}
1.00
0.50
0.30
0.20
0.10
0.05
0.03
0.02
0.01
0
60
120
Time, minutes
Composite Curve
Fast Compartment
Slow Compartment

APPENDIX 10D: Mean growth hormone kinetic curve for heifers adapted to a cold environment.


GH Concentration/Dose x 10^4
1.00
0.50
0.30
0.20
0.10
0.05
0.03
0.02
0.01
0
60
120
Time, minutes
Composite Curve
Fast Compartment
Slow Compartment

APPENDIX 11: Tables corresponding to Figures III-2 to III-7 presented in Chapter III.

APPENDIX 11A: Effect of insulin injection on plasma glucose concentration (mg mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means.

TIME AFTER INJECTION (MINUTES)	WARM	COLD
0	0.883	0.897
1	0.879	0.890
2	0.886	0.912
3	0.865	0.897
4	0.835*	0.885
6	0.798***	0.838**
10	0.722***	0.744***
15	0.632***	0.643***
20	0.555***	0.560***
25	0.507***	0.500***
30	0.464***	0.477***
40	0.414***	0.423***
50	0.443***	0.440***
60	0.483***	0.496***
75	0.564***	0.607***
90	0.643***	0.689***
105	0.691***	0.768***
120	0.747***	0.827***
150	0.846***	0.878
180	0.879***	0.932
Pooled SEM	0.013	0.013

In a column, if the sample is significantly different than time 0 (baseline): *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$

ANOVA TABLE OF MAIN EFFECTS

Temperature	P<0.001
Time	P<0.001
Temperature x Time	P=0.073
Pooled SEM for Time	0.010

APPENDIX 11B: Effect of insulin injection on plasma glucagon concentration (pg mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means.

TIME AFTER INJECTION (MINUTES)	WARM	COLD
0	217.7	258.5
1	224.6	251.0
2	204.2	258.0
3	218.9	250.9
4	208.2	237.2
6	219.1	250.6
10	228.4	283.2
15	262.2	345.4^{*}
20	272.4	350.1^{*}
25	273.6	359.9^{**}
30	281.3	378.7^{**}
40	395.3^{***}	504.0^{***}
50	608.5^{***}	667.2^{***}
60	548.4^{***}	660.3^{***}
75	591.4^{***}	578.1^{***}
90	389.2^{***}	389.4^{***}
105	280.0	292.4
120	243.7	255.6
150	216.3	230.8
180	199.6	221.4
Pooled SEM	30.3	26.8

In a column, if the sample is significantly different than time 0 (baseline): *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$

ANOVA TABLE OF MAIN EFFECTS	
Temperature	P<0.001
Time	P<0.001
Temperature x Time	Not Significant
Pooled SEM for Time	20.4

APPENDIX 11C: Effect of insulin injection on plasma growth hormone concentration (ng mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means.

TIME AFTER INJECTION (MINUTES)	WARM	COLD
0	4.821	4.626
1	4.995	4.860
2	5.355	4.764
3	5.436	4.749
4	5.229	4.790
6	5.305	4.465
10	4.776	4.709
15	4.831	5.036
20	5.125	5.104
25	5.247	5.537
30	5.187	5.666
40	5.534	6.041^{**}
50	5.368	5.931^{*}
60	5.691	5.263
75	5.776	6.031^{*}
90	6.664^{***}	6.071^{*}
105	7.228^{***}	6.269^{**}
120	6.260^{**}	6.164^{**}
150	6.205^{**}	5.967^{*}
180	6.496^{**}	5.917^{*}
Pooled SEM	0.358	0.373

In a column, if the sample is significantly different than time 0 (baseline): *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$

ANOVA TABLE OF MAIN EFFECTS	
Temperature	Not Significant
Time	P<0.001
Temperature x Time	Not Significant
Pooled SEM for Time	0.331

APPENDIX 11D: Effect of growth hormone injection on plasma glucose concentration (mg mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. (Only data from Period 2 shown).

TIME AFTER INJECTION (MINUTES)	WARM	COLD
0	0.831	0.904
1	0.808	0.874
2	0.815	0.900
3	0.839	0.924
4	0.830	0.921
6	0.850	0.929
10	0.835	0.932
15	0.842	0.915
20	0.832	0.905
25	0.809	0.850^{**}
30	0.783^{*}	0.875
40	0.776^{**}	0.885
50	0.797^{*}	0.897
60	0.802	0.872
75	0.803	0.869^{*}
90	0.835	0.929
105	0.828	0.921
120	0.854	0.917
150	0.865^{*}	0.926
180	0.873^{*}	0.940^{*}
Pooled SEM	0.012	0.012

In a column, if the sample is significantly different than time 0 (baseline): *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$

ANOVA TABLE OF MAIN EFFECTS	
Temperature	$P<0.05$
Time	$P<0.001$
Temperature x Time	Not Significant
Pooled SEM for Time	0.008

APPENDIX 11E: Effect of growth hormone injection on plasma insulin concentration (ng mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. (Only data from Period 2 shown).

TIME AFTER INJECTION (MINUTES)	WARM	COLD
0	1.786	2.544
1	1.924	2.442
2	2.008	2.796
3	2.016	2.888
4	2.040	2.902
6	2.620**	2.820
10	2.568**	3.594*
15	2.130	3.210
20	1.750	2.208
25	1.360	1.620*
30	1.108*	1.728
40	1.312	2.274
50	1.846	2.074
60	1.340	1.880
75	1.530	2.174
90	1.374	2.030
105	1.292	2.112
120	1.818	2.826
150	1.614	2.402
180	2.230	3.010
Pooled SEM	0.186	0.320

In a column, if the sample is significantly different than time 0 (baseline): *: P<0.05, **: P<0.01, ***: P<0.001

ANOVA TABLE OF MAIN EFFECTS	
Temperature	Not Significant
Time	P<0.001
Temperature x Time	Not Significant
Pooled SEM for Time	0.185

APPENDIX 11F: Effect of growth hormone injections at time o hours and time 24 hours on serum insulin-like growth factor I concentration (ng mL^{-1}) in heifers adapted to warm and cold environments. Means are least squares means. (Only data from Period 2 shown).

TIME AFTER INJECTION (HOURS)	WARM	COLD
0	249.6	308.5
6	277.5	338.6
12	276.9	342.9
18	276.8	335.9
24	268.0	327.6
30	318.5**	335.2
36	311.1**	364.9**
42	315.7**	380.7***
48	278.9	333.9
54	283.1	330.4
60	273.2	360.7*
66	266.6	373.3**
72	247.7	372.1**
Pooled SEM	16.1	13.8

In a column, if the sample is significantly different than time 0 (baseline): *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$

ANOVA TABLE OF MAIN EFFECTS	
Temperature	P=0.08
Time	P<0.01
Temperature x Time	P=0.10
Pooled SEM for Time	10.6